Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 321 (2004) 241–246
BBRC
www.elsevier.com/locate/ybbrc

Carbon monoxide exposure in rat heart: evidence for two modes of toxicity

Ashvin P. Patel [a,b], A. John Moody [a,*], J. Robert Sneyd [b], Richard D. Handy [a]

[a] School of Biological Sciences, University of Plymouth, Plymouth PL4 8AA, Devon, UK
[b] Peninsula Medical School, Tamar Science Park, Derriford, Plymouth PL6 8BX, Devon, UK

Received 23 June 2004

Abstract

Rat hearts were perfused for 30 min with buffer equilibrated with CO. Mean data for hearts exposed to 0.01% CO show a 15% decrease in heart rate (HR) during exposure followed by recovery, with a further 17% decrease post exposure. Examination of time courses from individual perfusions shows that in 10 hearts exposed to 0.01% CO HR responded in different ways: no response (5 hearts); decrease during exposure followed by recovery (3 hearts); and decrease post exposure (2 hearts). There was a strong association between CO-induced HR decrease and release of creatine kinase into the perfusate, both of which were not prevented by the antioxidants, ascorbate, and Trolox C. Perfusate flow rate declined post exposure (4.9% and 8.9% with 0.01% and 0.05% CO, respectively) and this was prevented by antioxidants. CO may have two, independent, cardiotoxic effects; these may be mediated by CO-induced elevation of oxidant production, H_2O_2 in one case and peroxynitrite in the other.

Keywords: Carbon monoxide; Oxidative stress; Rat heart; Antioxidants; Heart rate

Carbon monoxide (CO) is the most common cause of fatal poisoning in many Western countries [1,2]. Acute exposure to CO, e.g., via the incomplete combustion of domestic gas is well known [3], but chronic exposure also occurs, e.g., via cigarette smoking [4]. CO can cause tissue hypoxia by binding with high affinity to haemoglobin, and indeed the presence of carboxyhaemoglobin (COHb) is a useful diagnostic tool for CO exposure [2]. However, levels of COHb during acute CO poisoning do not correlate well with clinical outcome [5], and so it is clear that the pathophysiology of CO involves additional intracellular mechanisms of toxicity [6].

In skeletal and cardiac muscle, CO can cause cellular hypoxia by binding with high affinity to myoglobin. Also, in many tissues there is potential for CO to cause pseudohypoxia by blocking the usage of oxygen via cytochrome-*c* oxidase [7]. This raises the possibility that CO could cause an ischaemia–reperfusion like injury [6], and indeed there is evidence of oxidative stress in rat brain following the cessation of CO exposure [8].

In our previous work we showed that exposure of isolated perfused rat hearts to CO caused a depletion of ventricular glutathione levels post exposure [9]. However, it was argued that this CO-induced oxidative stress could not arise from an ischaemia–reperfusion like injury because the levels of CO used were insufficient to cause either pseudohypoxia or hypoxia. The work described here is an extension of our previous study. Here we describe the effects of CO on heart rate (HR) and coronary flow rate in isolated perfused rat hearts. In some hearts, CO induced a decrease in HR that is strongly associated with release of creatine kinase into the perfusate, whereas all hearts showed a decrease in perfusate flow rate immediately post exposure to CO that is prevented by inclusion of a combination of antioxidants, ascorbate and Trolox C, in the perfusion buffer. The evidence suggests that

* Corresponding author. Fax: +44-1752-232-970.
E-mail address: jmoody@plymouth.ac.uk (A.J. Moody).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.06.124

the cardiotoxicity of CO may arise from two, independent, mechanisms.

Materials and methods

Adult male Sprague–Dawley rats (262 ± 30 g body weight; mean ± SD, $n = 35$) were maintained with free access to food and water. All experiments were conducted in accordance with ethical approval. Unless otherwise stated, reagents were obtained from Sigma (Poole, UK).

Beating rat hearts (1.48 ± 0.15 g; mean ± SD, $n = 35$) were rapidly excised following anaesthesia with 60 mg intraperitonial sodium pentobarbital (Sagatal, May and Baker, Dagenham, UK) and perfused (at 36.9 °C) in a non-recirculating Langendorff apparatus at 6 ml/min/g wet weight of heart as described previously [10]. The perfusion buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.8 mM $CaCl_2$, 20 mM $NaHCO_3$, and 11 mM glucose) was constantly gassed with 21% oxygen, 5% carbon dioxide (balanced with nitrogen) to pH 7.4.

Hearts were perfused with buffer for about 10 min to wash out blood and to ensure that the HR was stable. A further 15 min perfusion, before CO exposure, was used to establish pre-exposure heart and perfusate flow rates. CO exposure was then started by perfusion with buffer that was saturated with 0%, 0.01% or 0.05% CO for 30 min, followed by a 90 min CO-free period. Gas mixtures (0–0.05% CO in 21% oxygen, 5% carbon dioxide and balanced with nitrogen) were supplied by BOC (Guildford, UK). In some perfusions antioxidants were included during the initial equilibration period and during the exposure to CO, but not during the 90 min of perfusion after CO exposure. These were 0.2 mM Trolox C ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; Fluka, Poole, UK) and 1 mM sodium ascorbate. The latter was added to the perfusion buffer immediately before the start of the perfusion. Note that the presence of ascorbate required greater buffering capacity in the perfusion buffer, so this was supplemented with 10 mM Hepes.

The activity of creatine kinase (CK) in 20 µl samples of perfusate was determined by standard enzyme-linked assay using a commercial kit (hexokinase/glucose-6-phosphate dehydrogenase based; Sigma product number 4720). Heart rates were determined by manual count over 30 s; measurements were carried out in triplicate. Perfusate flow rates were measured by collecting timed perfusate fractions and dividing the volume collected by the time taken to collect it.

SPSS 11.5 for Windows (SPSS) was used to generate pairwise comparisons of the HRs at different times within the one-way ANOVA GLM repeated measures procedure. Statgraphics Plus 5.1 (Statistical Graphics) was used to carry out cluster analysis on the HR data. The K-means method was used, with Euclidean distance as a measure of similarity; in this method the number of clusters is defined beforehand. It was used here as an objective way of grouping hearts according to their responses to CO exposure based on response types identified by visual inspection of the data.

Results

Effects of CO on heart rate

Fig. 1 shows the effect of exposure to 0.01% CO on HR. In controls, where the hearts were perfused with CO-free buffer throughout, there was no significant change in HR (repeated measures ANOVA, $P > 0.05$ for all pairwise comparisons of the HR at −2.5 min with HRs at other times). However, 0.01% CO induced a decrease in HR, which reached a minimum of 85% of the pre-exposure rate by 7.5 min of exposure (significant difference between the rates at −2.5 min and 7.5 min; $P = 0.042$). The HR then recovered over the rest of the exposure, and was close to the pre-exposure rate by the end of the exposure. After a lag of 10–15 min post exposure there was a second decrease in HR of similar magnitude to that seen during the exposure (significant difference between the rates at −2.5 min and 115 min; $P = 0.043$).

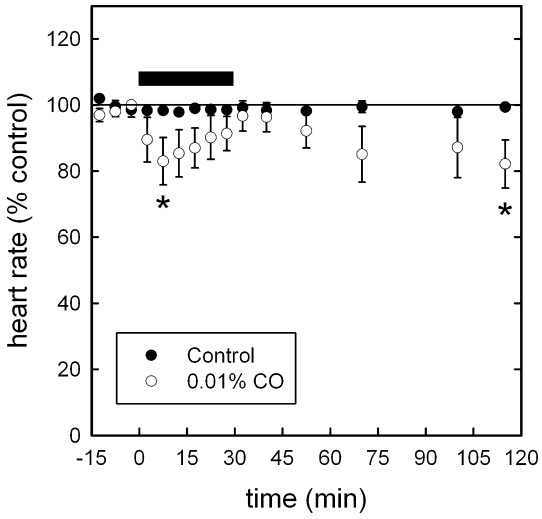

Fig. 1. Effect of 30 min exposure to 0.01% CO (black bar) on the HR of isolated perfused rat hearts, followed by a period of perfusion with CO-free medium (see Materials and methods for details). Mean values ± SEM are shown; $n = 3$ for the control (no CO exposure) and $n = 10$ for the exposure to CO. The asterisks indicate significant differences ($P < 0.05$) versus the HR at −2.5 min. The mean heart rate at −2.5 min was 146 ± 40 min^{-1} (±SD, $n = 13$).

It was apparent, from examination of HR time courses from individual perfusions, that the hearts responded in several ways to exposure to 0.01% CO, and that the two periods of HR decrease seen in the mean data in Fig. 1, i.e., during CO exposure and post CO exposure, were not associated with the same hearts. When cluster analysis (see Materials and methods) was carried out on the HR data from 7.5, 12.5, and 17.5 min (i.e., during the exposure) two types of response to CO exposure were revealed. In one group of hearts (Type 1, 3 hearts) there was a CO-induced decrease in HR during the exposure and in the other (Type 2, 7 hearts) this response was absent (Fig. 2A). Furthermore, hearts that showed the Type 2 response could be separated into two subgroups using cluster analysis of the HR data from 70, 100, and 115 min (i.e., post exposure): Type 2A, 2 hearts which showed a CO-induced decrease in HR post exposure, and Type 2B, 5 hearts in which this was absent (Fig. 2A). Hence, there were five hearts (Type 2B) which showed no CO-induced change in HR throughout the perfusion.

Eight of the hearts exposed to 0.01% CO showed periods of arrhythmia either during or after the CO

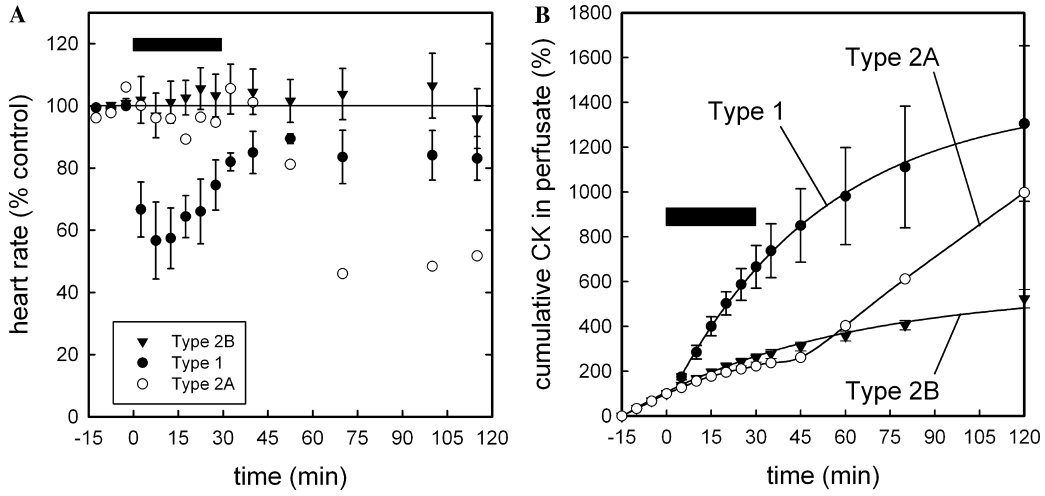

Fig. 2. Effects of exposure to 0.01% CO on the HR (A) and CK release (B) from perfused isolated rat hearts. (B) Cumulative CK in the perfusate expressed as a percentage of relative to the amount accumulated in the 15 min before the CO exposure (see Materials and methods). The mean cumulative CK activity in these hearts, at $t = 0$, was 3.74 ± 1.84 IU/g wet tissue, means ± SD, $n = 10$. The data from the 10 hearts have been grouped as described in the text. In both panels the black bar marks the period of exposure to CO. The data shown are means ± SEM as appropriate: $n = 3$ for Type 1, $n = 2$ for Type 2A, and $n = 5$ for Type 2B.

exposure, or at both times. Two hearts exposed to 0.01% CO showed no arrhythmia; these showed a Type 2B response in terms of HR as defined above, i.e., they showed no CO-induced changes in HR.

In hearts exposed to 0.05% CO there was a slight indication of a decrease in HR during the exposure (Fig. 3A) relative to the rate at −2.5 min, but this was not statistically significant (repeated measures ANOVA, $P > 0.05$ for all pairwise comparisons of the HR at −2.5 min with HRs at other times). However, all hearts exposed to 0.05% CO showed periods of arrhythmia either during the exposure or after, or both during and after.

Effect of CO on coronary flow

Coronary flow was monitored during and after exposure of perfused rat hearts to 0.01% or 0.05% CO. Table 1 shows the mean perfusate flow rates during exposure (0–30 min) and post exposure (30–120 min) expressed as percentages of the flow rates pre exposure (−15–0 min). CO at 0.01% or 0.05% caused no significant change in perfusate flow rate during the exposure, but there was a dose-dependent decline post exposure of 4.9% and 8.9% with 0.01% and 0.05% CO, respectively. In control hearts over the same period the decline was only 1.2%. In addition to the decline in

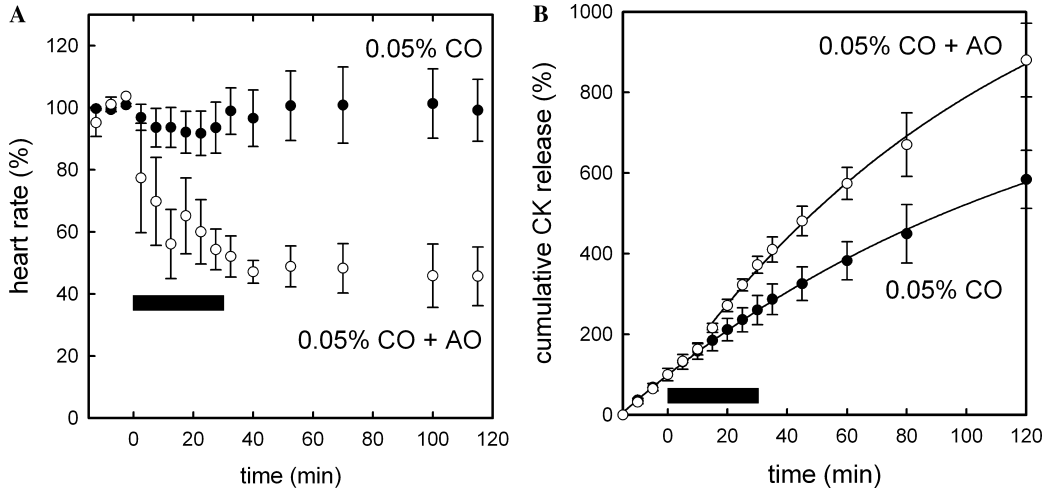

Fig. 3. Effects of the antioxidants ascorbate and Trolox C (AO) on HR (A) and CK release (B) from perfused isolated rat hearts exposed to 0.05% CO. The cumulative CK in the perfusate is expressed as a percentage of relative the amount accumulated in the 15 min before the CO exposure (see Materials and methods). In both panels the black bar marks the period of exposure to CO. The data shown are means ± SEM: $n = 5$ for the hearts exposed to 0.05% CO only and $n = 4$ for those exposed to 0.05% CO plus antioxidants.

Table 1
The effects of CO exposure on perfusate flow rate

CO exposure (%)	During (%)	Post (%)	*n*
0	99.9 ± 0.4	98.7 ± 0.4	3
0 + AO	99.5 ± 1.1	98.7 ± 1.5	3
0.01	98.9 ± 0.6	94.0 ± 0.5[a,b]	10
0.05	99.3 ± 0.6	90.5 ± 2.1[a,b,c,d]	6
0.05 + AO	100.2 ± 0.9	97.9 ± 1.0	5

The initial perfusate flow rate for each heart was set at 6 ml/min/g wet weight of heart. Data shown are means ± SEM (*n* values as indicated) expressed as a percentage of the pre CO flow rate.
[a] Significantly different to 'during;' 95% confidence level.
[b] Significantly different to '0%, post;' 95% confidence level.
[c] Significantly different to '0.05% + AO, post;' 95% confidence level.
[d] Significantly different to '0.01%, post;' 95% confidence level.

perfusate flow immediately post exposure, hearts exposed to CO showed signs of swelling particularly of the vasculature close to the cannula.

Effect of antioxidants on CO-induced changes in HR and coronary flow

Inclusion of a combination of water-soluble antioxidants, ascorbate and Trolox C, in the perfusion buffer in the equilibration period before exposure and during the exposure to 0.05% CO did not prevent the CO-induced decrease in HR (Fig. 3A). All four hearts exposed to 0.05% CO in the presence of antioxidants showed a decline in HR immediately after the start of the CO exposure. Moreover, there was no recovery of the HR in this case.

Despite the lack of a protective effect against HR decrease the antioxidants did, however, have a significant protective effect on the CO-induced decline in perfusate flow rate seen with 0.05% CO (Table 1), such that this fell by only 2.0%, not significantly different to the decrease (0.8%) seen in the control (0% + AO, Table 1).

Heart rate changes and creatine kinase release

In our previous report [9] we noted that isolated perfused rat hearts exposed to 0.01% CO showed different responses in terms of the release of CK into the perfusate, an indication of CO-induced tissue damage. In some hearts there was no CO-induced damage, whereas in others there was damage either immediately after the start of exposure or lagging 10–15 min after the end of the exposure. Fig. 2B shows the cumulative perfusate CK data for hearts exposed to 0.01% CO grouped according to the cluster analysis of the HR data described earlier. It is clear from comparison of Figs. 2A and B that there is a strong association between the occurrence of CO-induced HR changes and of CO-induced CK release. A similar relationship can be seen in Figs. 3A and B: hearts exposed to 0.05% CO showed little or no change in HR (Fig. 3B) and correspondingly no CO-induced CK release (Fig. 3B) whereas those exposed to 0.05% CO in the presence of antioxidants showed a decrease in HR (Fig. 3A) and a corresponding increase in CK release (Fig. 3B) although interestingly there was a lag of about 10 min before this occurred.

Discussion

In the work described here the effects of exposure of isolated perfused rat hearts to CO on HR and perfusate flow were examined. The data presented suggest that CO has at least two, possibly independent, toxic effects. There was a dose-dependent decrease in perfusate flow post exposure to CO, which was seen in all hearts exposed to CO and which is prevented by inclusion of the antioxidants ascorbate and Trolox C in the perfusion buffer (Table 1). However, there were also CO-induced decreases in HR in some, but not all hearts, either immediately after the start of the exposure or lagging 10–15 min after the end of the exposure (Fig. 2), that do not appear to be prevented by ascorbate and Trolox C in the perfusion buffer (Fig. 3).

The idea that CO has two independent effects on perfused rat hearts is supported by the data on perfusate CK levels (Figs. 2 and 3) and the data on ventricular glutathione in our previous report [9]. Some of the hearts exposed to CO showed episodes of tissue damage as indicated by the release of CK into the perfusate, and it is clear that there is a strong association between CO-induced CK release and CO-induced HR decrease (Figs. 2 and 3). For example, those hearts that showed CO-induced CK release immediately after the start of the exposure to 0.01% CO also showed a concomitant decrease in HR (Type 1 in Fig. 2). In contrast, we showed previously [9] that all hearts exposed to 0.01% or 0.05% CO showed a severe decline in ventricular glutathione post exposure, and that this could be mostly prevented by ascorbate/Trolox C.

The HR data suggest that the CO levels used here (0.01% and 0.05% CO) were insufficient to cause tissue hypoxia (via binding to myoglobin) or pseudohypoxia (via inhibition of cytochrome-*c* oxidase). If the CO had an effect analogous to ischaemia then we would expect to see a progressive decline in HR in *all* hearts during the exposure. For example, Allibardi et al. [11] reported a decline in HR to less than 40% of the control after 30 min of low-flow ischaemia in isolated rat heart perfusions whereas Hammad et al. [12] observed a progressive decline in HR during global ischaemia with complete cessation of beating after 15 min. However, not only did some hearts show no CO-induced HR change at all, but those that showed a CO-induced decrease during the 0.01% CO exposure also showed almost complete recovery by the end of the 30 min exposure (Fig. 2). Neither observation is consistent with CO causing substantial tissue hypoxia/pseudohypoxia,

providing further support for our previous conclusion that glutathione depletion post CO exposure could not be caused by oxidative stress resulting from an ischaemia–reperfusion-like injury [9].

Does CO induce H_2O_2 production post exposure?

Although we rule out an ischaemia–reperfusion-like injury in our experiments, nevertheless, the severe decline in glutathione [9], together with the antioxidant protection of the decrease in perfusate flow described here (Table 1), suggests the generation of an oxidant post CO exposure. A candidate for this oxidant is hydrogen peroxide (H_2O_2) as it is known that perfusion of isolated rat hearts with H_2O_2 causes glutathione depletion [13]. This could occur through oxidation of reduced glutathione (GSH) and the subsequent loss of oxidised glutathione (GSSG) into the perfusate [14]. In the myocardium both glutathione peroxidases and myoglobin [15] could catalyse the oxidation of GSH by H_2O_2. Dulchavsky et al. [13] found that there was a substantial release of glutathione (GSH + GSSG) but not LDH into the perfusate from hearts perfused for 30 min with 200 μM H_2O_2. LDH release was only observed when higher concentrations of H_2O_2 were used. In our hands retrograde perfusion of rat hearts with 430 μM H_2O_2 caused no release of LDH until about 15 min into the perfusion (Patel, A.P., Moody, A.J. Sneyd, J.R. and Handy, R.D., unpublished observation). Hence, exposure to H_2O_2 can cause a depletion of glutathione, but not necessarily release of LDH. This is consistent with our finding that there is no correlation between CO-induced LDH or CK release and glutathione loss [9].

Can low CO:O_2 ratios lead to the generation of super oxide or a derivative of superoxide?

As noted above, there is an association between CO-induced CK release and decrease in HR (Figs. 2 and 3). It is possible that both these are mediated by superoxide ($O_2^{\bullet-}$) or a derivative of $O_2^{\bullet-}$, for example peroxynitrite ($ONOO^-$) [16]. Exposure of isolated and in situ mammalian hearts to $O_2^{\bullet-}$ generated exogenously, e.g., by perfusion with xanthine/xanthine oxidase, causes a decline in contractility, including a decrease in HR, which can mostly be eliminated by superoxide dismutase [17]. Perfusion of isolated rat hearts with H_2O_2 is also known to cause a depression in HR, and eventual heart failure [13,18,19] but heart contractility is much more sensitive to $O_2^{\bullet-}$ than H_2O_2. For example, in the work by Prasad et al. [17] the maximum $[O_2^{\bullet-}]$ that could have been present in the perfusion buffer was about 23 μM, i.e., 2× the initial [xanthine] in the perfusion buffer. Most studies in which rat hearts have been perfused with H_2O_2 involve concentrations about an order of magnitude higher than this.

The mechanism by which $O_2^{\bullet-}$ affects HR may involve $O_2^{\bullet-}$-induced Ca^{2+} release from the sarcoplasmic reticulum via ryanodine-sensitive Ca^{2+} channels [20]; Ca^{2+} release from sarcoplasmic reticulum contributes to pacemaking in the sino-atrial node [21].

What could be the sources of CO-induced oxidant production?

As discussed above, it is possible to rationalise the observed cardiotoxic effects of CO in terms of production of H_2O_2 and $O_2^{\bullet-}$ or a derivative of $O_2^{\bullet-}$, although it is less clear how CO could induce their production.

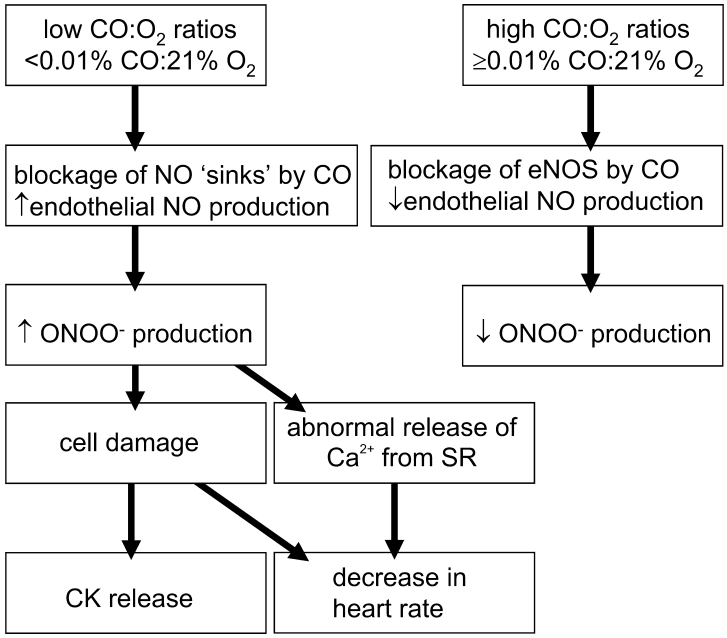

Fig. 4. Schematic diagram showing hypothetical connection between low CO:O_2 ratios, CK release, and depression of HR.

On one hand, the putative H_2O_2 production occurs post CO exposure, and so presumably involves the accumulation of a substrate/precursor during the CO exposure. On the other hand, the putative $O_2^{\bullet-}$ production must be transient, since the toxic effects are mostly transient (Fig. 2). In our previous report [9] we speculated that low CO:O_2 ratios, seen transiently at the start of the CO exposure, and again after the end of the exposure, could be responsible for this. Thom et al. [22] showed that exposure of endothelial cells to low levels of CO (10–110 nM, i.e., in part below the lowest concentration used here) caused an increase in steady-state nitric oxide (NO) and NO-derived oxidants, e.g., peroxynitrite. They suggested that competition from CO for intracellular NO-binding sites contributes to elevation of the NO levels. Another possibility is inhibition by CO of NO detoxification [23,24]. Although low concentrations of CO can stimulate endothelial NO production, higher concentrations could inhibit NO production by blocking the cytochrome P450-like oxygenase domain of endothelial nitric oxide synthase (eNOS) (e.g., [25]), thereby providing an explanation for the transiency of some of the cardiotoxic effects of CO described here (Fig. 4).

In conclusion, the evidence presented in this paper together with that in our previous report [9] suggests that CO has two, possibly independent, cardiotoxic effects. We propose that these effects are mediated by the CO-induced production of the oxidants, H_2O_2 and peroxynitrite ($ONOO^-$). Elevated peroxynitrite production could arise from perturbation of endothelial NO metabolism.

Acknowledgments

The authors thank the following bodies for their financial support: the Diving Diseases Research Centre (DDRC), Plymouth, UK; the British Antarctic Survey (BAS), and the British Journal of Anaesthesia (BJA).

References

[1] K.R. Hardy, S.R. Thom, Pathophysiology and treatment of carbon monoxide poisoning, J. Toxicol. Clin. Toxicol. 32 (1994) 613–629.
[2] M. Turner, M.R. Hamilton-Farrell, R.J. Clark, Carbon monoxide poisoning: an update, J. Accid. Emerg. Med. 16 (1999) 92–96.
[3] M.R. Hamilton-Farrell, British Hyperbaric Association carbon monoxide database, 1993–1996, J. Accid. Emerg. Med. 16 (1999) 98–103.
[4] R.M. Pittilo, Cigarette smoking, endothelial injury and cardiovascular disease, Int. J. Exp. Path. 81 (2000) 219–230.
[5] D.M. Norkool, J.N. Kirkpatrick, Treatment of acute carbon monoxide poisoning with hyperbaric oxygen: a review of 115 cases, Ann. Emerg. Med. 14 (1985) 1168–1171.
[6] C.A. Piantadosi, Toxicity of carbon monoxide: haemoglobin vs. histotoxic mechanisms, in: D.G. Penney (Ed.), Carbon Monoxide, CRC Press, Boca Raton, 2003, pp. 163–186.
[7] R.F. Coburn, Mechanisms of carbon monoxide toxicity, Prev. Med. 8 (1979) 312–322.
[8] J. Zhang, C.A. Piantadosi, Mitochondrial oxidative stress after carbon monoxide hypoxia in the rat brain, J. Clin. Invest. 90 (1992) 1193–1199.
[9] A.P. Patel, A.J. Moody, R.D. Handy, J.R. Sneyd, Carbon monoxide exposure in rat heart: glutathione depletion is prevented by antioxidants, Biochem. Biophys. Res. Commun. 302 (2003) 392–396.
[10] N. Haramaki, H. Assadnazari, G. Zimmer, V. Schepkin, L. Packer, The influence of vitamin E and dihydrolipoic acid on cardiac energy and glutathione status under hypoxia-reoxygenation, Biochem. Mol. Biol. Int. 37 (1995) 591–597.
[11] S. Allibardi, G. Merati, S. Chierchia, M. Samaja, Atenolol depresses post-ischaemic recovery in the isolated rat heart, Pharmacol. Res. 39 (1999) 431–435.
[12] D. Hammad, S.A. Omar, A. Husain, M.M. Essa, Cardioprotective effects of ascorbic acid on the injury induced by ischaemia–reperfusion in isolated mammalian heart, Med. Sci. Res. 25 (1997) 69–71.
[13] S.A. Dulchavsky, S.B. Davidson, W.J. Cullen, T.P.A. Devasagayam, L.N. Diebel, S. Dutta, Effects of deferoxamine on H_2O_2-induced oxidative stress in isolated rat heart, Basic Res. Cardiol. 91 (1996) 418–424.
[14] T. Ishikawa, H. Sies, Cardiac transport of glutathione disulfide and S-conjugate. Studies with isolated perfused rat heart during hydroperoxide metabolism, J. Biol. Chem. 259 (1984) 3838–3943.
[15] D. Galaris, E. Cadenas, P. Hochstein, Glutathione-dependent reduction of peroxides during ferryl- and met-myoglobin interconversion: a potential protective mechanism in muscle, Free Radic. Biol. Med. 6 (1989) 473–478.
[16] S.A. Gupte, T. Okada, Prostaglandins and nitric oxide mediate superoxide-induced myocardial contractile dysfunction in isolated rat hearts, J. Mol. Cell. Cardiol. 33 (2001) 1107–1117.
[17] K. Prasad, J. Kalra, L. Bharadwaj, Cardiac depressant effects of oxygen free radicals, Angiology 44 (1993) 257–270.
[18] T. Onodera, G. Takemura, T. Oguro, M. Ashraf, Effect of exogenous hydrogen peroxide on myocardial function and structure in isolated rat heart, Can. J. Cardiol. 8 (1992) 989–997.
[19] G.J. Harrison, L.R. Jordan, R.J. Willis, Deleterious effects of hydrogen peroxide on the function and ultrastructure of cardiac muscle and the coronary vasculature of perfused rat hearts, Can. J. Cardiol. 10 (1994) 843–849.
[20] E. Okabe, K. Kuse, T. Sekishita, N. Suyama, K. Tanaka, H. Ito, The effect of ryanodine on oxygen free radical-induced dysfunction of cardiac sarcoplasmic reticulum, J. Pharmacol. Exp. Ther. 256 (1991) 868–875.
[21] L. Rigg, D.A. Terrar, Possible role of calcium release from the sarcoplasmic reticulum in pacemaking in guinea-pig sino-atrial node, Exp. Physiol. 81 (1996) 877–880.
[22] S.R. Thom, Y.A. Xu, H. Ischiropoulos, Vascular endothelial cells generate peroxynitrite in response to carbon monoxide exposure, Chem. Res. Toxicol. 10 (1997) 1023–1031.
[23] P.R. Gardner, L.A. Martin, D. Hall, A.M. Gardner, Dioxygen-dependent metabolism of nitric oxide in mammalian cells, Free Radic. Biol. Med. 31 (2001) 191–204.
[24] C. Griffiths, B. Yamini, C. Hall, J. Garthwaite, Nitric oxide inactivation in brain by a novel O_2-dependent mechanism resulting in the formation of nitrate ions, Biochem. J. 362 (2002) 459–464.
[25] J.M. Griscavage, N.E. Rogers, M.P. Sherman, L.J. Ignarro, Inducible nitric oxide synthase from a rat alveolar macrophage cell line is inhibited by nitric oxide, J. Immunol. 151 (1993) 6329–6337.