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Catalase activity in coronary artery endothelium protects smooth muscle against peroxide damage

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Abstract

Cyclopiazonic acid contracts pig coronary artery de-endothelialized rings, and pretreating the rings with hydrogen peroxide (H_2O_2) inhibits this contraction ($IC_{50} = 0.097 \pm 0.013$ mM). We used the cyclopiazonic acid contraction to test the novel hypothesis that endothelium can protect underlying smooth muscle against luminal H_2O_2. We perfused the arteries with Krebs' solution containing 0.3 or 1 mM H_2O_2, removing endothelium from the arteries either before or after the perfusion. We then cut rings from them to monitor their contraction to 10 μM cyclopiazonic acid in a H_2O_2-free solution. The inhibition of the cyclopiazonic acid contraction by perfusion with H_2O_2 was significantly less when endothelium was removed after the perfusion than when it was removed before it. The specific activity of catalase in post-nuclear supernatants from freshly isolated endothelium (14.1 ± 2.7 μmol/min/mg protein) was 17 ± 3-fold greater than in those from smooth muscle (0.83 ± 0.22 μmol/min/mg protein). Thus endothelium contained high catalase activity and protected the underlying smooth muscle against luminal peroxide.

Keywords: Ischemia–reperfusion; Cyclopiazonic acid; Oxidative stress; Free radical; Reactive oxygen species

1. Introduction

Reactive oxygen species are the main source of cardiovascular damage in an ischemia–reperfusion injury (Singal et al., 1998). In particular, the reperfused ischemic tissues accumulate large amounts of reactive oxygen species, especially peroxide, superoxide and perhydroxyl radicals. The high concentrations of reactive oxygen species may cause extensive damage to cells, including the depletion of ascorbate and reduced glutathione, lipid peroxidation, oxidation of protein sulfhydryl groups, elevated intracellular Ca^{2+} levels, and DNA breakage (Darley-Usmar and Halliwell, 1996; Halliwell, 1996). The lipid peroxidation can, in turn, increase plasma membrane permeability, breakdown of transmembrane ion gradients, and inhibit cellular metabolic processes through a variety of reactions. For example, the sarco/endoplasmic reticular (SERCA) Ca^{2+}-pump in the coronary artery smooth muscle is extremely sensitive to reactive oxygen species (Grover and Samson, 1989; Grover et al., 1992). This damage to the SERCA pump accompanies a loss of reactivity of the vessels in response to various agents (Grover et al., 1995, 1997).

Endothelium is involved in several processes including inflammation, platelet aggregation and thrombosis, fibrinolysis, angiogenesis, and regulation of vascular tone (Cosentino and Luscher, 1997; Luscher and Barton, 1997). In coronary artery, the peroxide resistance of the SERCA pump endothelium is greater than that for the smooth muscle SERCA pump (Grover and Samson, 1997). The artery smooth muscle expresses mainly the gene SERCA2 while endothelium may express both SERCA2 and SERCA3 (Wu et al., 1995). The peroxide resistance of SERCA3 protein is slightly greater than that of SERCA2 protein (Grover et al., 1997). However, the peroxide resistance of the SERCA pump in endothelium is much greater than that expected from its SERCA3 expression (Grover and Samson, 1997). These results suggest that endothelium has protective mechanisms against reactive oxygen species and that these may allow endothelium to perform an additional function — to protect smooth muscle from damage by reactive oxygen species. Here, we test the novel hypothesis that the endothelium protects underlying smooth muscles against luminal peroxide in the coronary artery.

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2. Experimental methods

2.1. Dissection and perfusion of the arteries

Pig hearts were obtained from the slaughter house Maple Leaf Meats (Burlington, Ontario, Canada) and placed immediately in an ice cold physiological saline solution at pH 6.4 (Grover and Samson, 1997). Cardiac muscle, fat and connective tissue were dissected away leaving the origins of the branch arteries intact. Major branches of arteries were ligated with surgical silk. Tygon tubing was then inserted into the origin of the arteries for perfusion. The perfusion apparatus consisted of several parts: a perfusion box which held the arteries, a peristaltic pump and a drip tray to keep the arteries moist with the Krebs' solution and to prevent a direct action of peroxide from the adventitious side. The arteries were perfused at 37°C with the Krebs' solution containing specified concentrations of H_2O_2. The perfusion pressure was kept constant to yield a flow of 5.88 ml/min per artery. Details of the perfusion apparatus are available from the authors upon request.

Endothelium was removed mechanically from the arteries by drawing a piece of cotton either before or after the perfusion as specified in the Results. The endothelium removal was confirmed occasionally by the absence of endothelium-dependent relaxation to bradykinin (10^{-7} M) (Grover and Samson, 1997). The arteries were perfused with Krebs' solution for 30 min followed by 30 min with Krebs' solution containing specified concentrations of peroxide. Peroxide was then removed by perfusing the arteries with Krebs' solution without peroxide for 60 min. After the perfusion the endothelium of the two arteries with intact endothelium during perfusion was removed. The arteries were cut into 3 mm-long rings. Two rings from the middle region of each artery were used for measurement of tension in organ baths and the mean value of their tension was the value used from one animal.

2.2. Contractility experiments

All the contractility measurements were carried out on de-endothelialized artery rings in organ baths containing oxygenated Krebs' solution at 37°C under an initial tension of 3 g which was readjusted 30 min later again to 3 g (Grover and Samson, 1997). The rings were equilibrated for 60 min and then their contraction with 60 mM KCl was monitored for 10 min. The tissues were washed 5 × with 5 ml normal Krebs' solution over a period of 5 min and were then allowed to equilibrate for 10 min. The KCl contraction was repeated. The rings were washed again 5 × with 5 ml normal Krebs' solution over a period of 5 min and then allowed to equilibrate for 30 min. The coronary artery rings were then contracted with the SERCA pump inhibitor 10 μM cyclopiazonic acid. This cyclopiazonic acid this concentration gives the maximum contraction. The tissues were blotted and weighed at the end of the experiment.

2.3. Catalase assays

For catalase assays, endothelial cells were freshly isolated typically from 10 pig coronary arteries as described previously and then placed in chilled 250 mM sucrose (Grover and Samson, 1989). The cells were separated by centrifugation at 50 g for 2 min and then homogenized in 250 mM sucrose using a glass/teflon homogenizer. The homogenate was centrifuged at $1000 \times g$ for 2 min to obtain the post-nuclear supernatant. Smooth muscle post-nuclear supernatant was prepared in 250 mM sucrose and catalase activity (Grover and Samson, 1989) was assayed at 20–22°C in 10 mM K-phosphate pH 7.8 as decrease in absorbance at 240 nm (extinction coefficient = 43.6 M^{-1} cm^{-1}).

2.4. Data analysis

Statistical analysis of the data were carried out by Analysis of Variance using the computer program Instat (Graphpad, USA). A P value < 0.05 was considered to be statistically significant. Curve fitting was carried out with FigP (Biosoft, USA).

3. Results

3.1. Peroxide treatment of artery rings in organ baths

Fig. 1 shows the effect of bathing de-endothelialized coronary artery rings with H_2O_2 on the subsequent contractions to 10 μM cyclopiazonic acid in peroxide-free Krebs' solution. The peroxide pretreatment inhibited the cyclopiazonic acid contractions with an IC_{50} value of

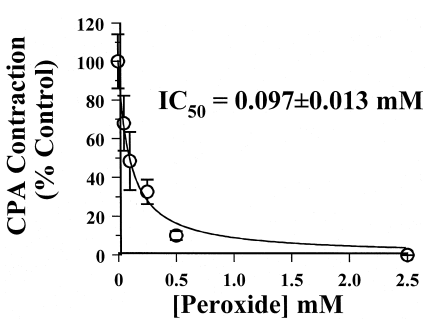

Fig. 1. Cyclopiazonic acid contraction of de-endothelialized coronary artery rings bathed in H_2O_2. The rings were treated with the specified concentrations of H_2O_2 in an organ bath, washed with the Krebs' solution, and their contraction to 10 μM cyclopiazonic acid was determined. The data are mean ± S.E.M. of the values obtained from a total of 38 pigs: 10 for 0 mM H_2O_2 and 3–8 for each of the other H_2O_2 concentrations. Best fit gave IC_{50} = 0.097 mM with an S.E.M. of 0.013 mM.

0.097 ± 0.013 mM. Contractions to 60 mM KCl were affected significantly only by the higher H_2O_2 concentrations (> 0.5 mM, not shown). In subsequent experiments, we determined if luminal peroxide would produce a similar inhibition of the cyclopiazonic acid contractions and if endothelium could protect the underlying smooth muscle from it.

3.2. Perfusion of arteries with peroxide and cyclopiazonic acid contractions

We asked three related questions: (a) does endothelium removal before the perfusion in H_2O_2-free solution itself affect the subsequent contractions, (b) does perfusion with H_2O_2 produce a similar damage to the subsequent cyclopiazonic acid contractions in peroxide-free solution, and (c) does endothelium protect smooth muscle against H_2O_2 in the perfusion solution? To answer these questions, we perfused arteries with Krebs' solution with or without H_2O_2 and removed the endothelium before or after the perfusion. In one set of experiments, we used four arteries per day, removing endothelium before perfusion from two and after the perfusion from the others. From each set, one artery was perfused with 0 mM and the other with 0.3 mM H_2O_2 in the Krebs' solution. During the contraction measurements, the arteries were always de-endothelialized and free of peroxide. In another set of experiments we followed the same protocol except that the peroxide concentration was 0 or 1 mM. Data from the two sets of experiments were pooled for the analysis.

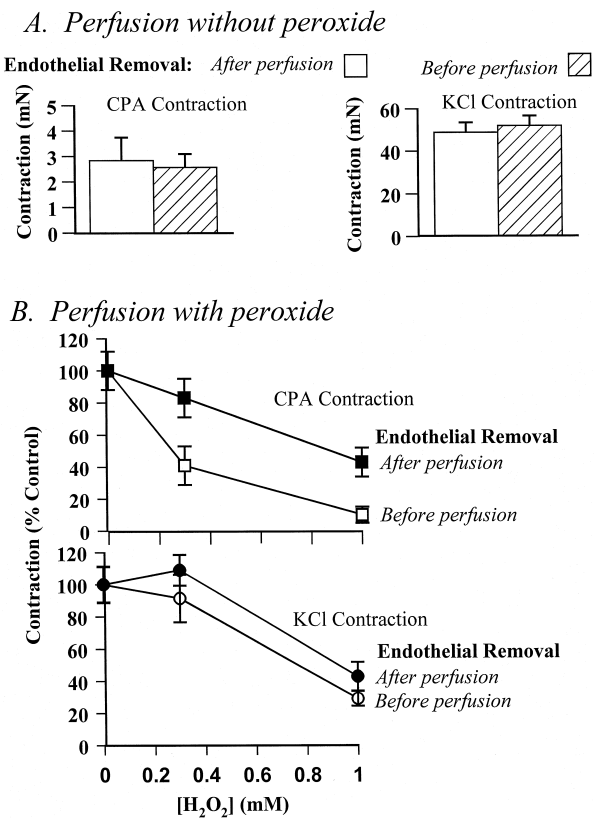

Fig. 2. Cyclopiazonic acid and KCl contractions of perfused arteries. (A) Arteries perfused without H_2O_2. Arteries were perfused without H_2O_2 with the endothelium removed before or after the perfusion. The data are mean ± S.E.M. of 21 animals for endothelium removed after the perfusion and 20 animals for the endothelium removed before the perfusion. The after vs. before groups did not differ significantly ($P > 0.05$) from each other for contractions with 10 μM cyclopiazonic acid or 60 mM KCl. (B) Arteries perfused with H_2O_2. Arteries were perfused with H_2O_2 with the endothelium removed before or after the perfusion. The data are from a total of 79 animals. The mean value of the contraction without H_2O_2 was determined and all the values were expressed as its percentage. The after vs. before groups differed significantly ($P < 0.05$) from each other for cyclopiazonic acid contractions at 0.3 and 1 mM H_2O_2 but not for KCl contractions ($P > 0.05$).

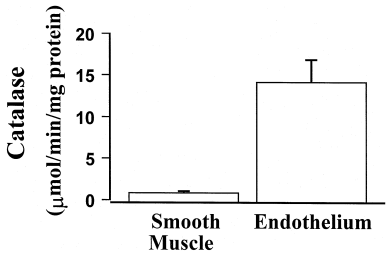

Fig. 3. Specific activity of catalase in smooth muscle and endothelium. In each experiment, the average values of catalase activity and protein concentration were computed from 3–4 measurements. Specific activity = average value of catalase activity/average value of protein. The data are mean ± S.E.M. of specific activities of catalase determined in four experiments ($n = 4$). The specific activity values for catalase in smooth muscle and endothelium differed significantly ($P < 0.05$) from each other, the latter being 17 ± 3-fold (mean ± S.E.M.) higher.

Fig. 2A is the perfusion control without H_2O_2. The force of contraction produced by KCl or did not differ significantly ($P > 0.05$) when endothelium was removed before the perfusion from that cyclopiazonic acid when it was removed after the perfusion.

Fig. 2B shows that perfusing the de-endothelialized arteries with H_2O_2 inhibited the subsequent cyclopiazonic acid contractions in a peroxide-free solution. Now, we asked the question whether it made a difference to the inhibition of the contractions by luminal peroxide as to when endothelium was removed — before the perfusion or after it. Fig. 2B shows that when endothelium was removed before the perfusion with 0.3 mM H_2O_2, the remaining cyclopiazonic acid contractions were $41 \pm 12\%$ of the control but if it was removed after the perfusion it was significantly greater ($83 \pm 12\%$, $P < 0.05$). Similarly, when 1 mM H_2O_2 was used, the remaining contractions were $10 \pm 5\%$ if endothelium was removed before and significantly greater ($43 \pm 9\%$, $P < 0.05$) when it was removed after. The contractions to KCl were lower after the pretreatment with 1 mM but not with 0.3 mM H_2O_2 (Fig. 2B). Since the perfusion with 0.3 mM H_2O_2 did not significantly affect the KCl contractions, we also analysed the data as cyclopiazonic acid:KCl contraction ratio in each tissue and arrived at the same conclusion. Based on the four methods of analysis, we concluded that endothe-

lium protected the underlying smooth muscle from luminal H_2O_2.

3.3. Catalase activities in endothelium and smooth muscle

Fig. 3 shows the results of catalase activity measurements in the post-nuclear supernatants prepared from freshly isolated endothelium and smooth muscle. The specific activity of catalase in the endothelium was 14.1 ± 2.7 μmol/min/g protein and in the smooth muscle, this value was 0.83 ± 0.22. Thus endothelium contained 17 ± 3 times higher specific activity of catalase than the smooth muscle.

4. Discussion

The Results section show that in pig coronary artery the endothelium protects the underlying smooth muscle against luminal peroxide and that the specific activity of catalase is 17 ± 3-fold higher in endothelium than in smooth muscle.

We have previously shown that H_2O_2 produces a transient contraction in the coronary artery (Grover et al., 1999). Incubating microsomes isolated from the smooth muscle with H_2O_2 damages the sarcoplasmic reticular Ca^{2+} pump preferentially over the plasma membrane Ca^{2+} pump (Grover et al., 1992). Cyclopiazonic acid inhibits the sarcoplasmic reticular Ca^{2+} pump in coronary artery smooth muscle permeabilized cells (Elmoselhi et al., 1995). In cultured cells in Ca^{2+}-free solutions, cyclopiazonic acid produces a transient increase in cytosolic Ca^{2+} but in Ca^{2+}-containing solution, this increase is sustained (Grover et al., 1995). Cyclopiazonic acid also produces a contraction in de-endothelialized coronary artery rings (Grover et al., 1997). Pretreating cells or tissues with peroxide inhibits the subsequent cyclopiazonic acid response. Thus, H_2O_2 may inhibit the cyclopiazonic acid response by damaging the sarcoplasmic reticular Ca^{2+} pump even though alternative mechanisms (Van Breemen et al., 1997) cannot be ruled out. The effects of H_2O_2 on the de-endothelialized arteries were similar whether the artery rings were bathed in H_2O_2 in an organ bath or if the arteries were perfused in the lumen with H_2O_2. The values of $59 \pm 12\%$ and $90 \pm 5\%$ for inhibition upon perfusion with 0.3 mM and 1 mM H_2O_2 (Fig. 2B), respectively, are consistent with the IC_{50} value of 0.097 ± 0.013 mM obtained by bathing the de-endothelialized artery rings in H_2O_2 (Fig. 1). The results presented here are also consistent with our previous report. The contractions of the artery to membrane depolarization with 60 mM KCl, which depend on Ca^{2+} entry via voltage operated Ca^{2+} channels, are affected by higher concentrations of H_2O_2 than those with angiotensin II or cyclopiazonic acid which depend on sarcoplasmic reticular Ca^{2+} (Grover et al., 1995).

The conclusion that endothelium protects the cyclopiazonic acid contraction in pig coronary arteries exposed to luminal H_2O_2 was independent of how the data were analysed and whether 0.3 or 1 mM H_2O_2 was used. The result that specific activity of catalase is much higher in endothelium than in smooth muscle provides a direct explanation for the protection, namely, catalase in the endothelium prevents H_2O_2 from reaching the underlying smooth muscle by decomposing it. However, as other antioxidant activities may be involved, it may not be the only mechanism. The endothelium preparation obtained in these experiments contains 10–15% smooth muscle as contamination (Shah et al., 1998). Hence the absolute specific activity of catalase in the endothelium may be slightly higher. It is not known if endothelium has higher levels of other antioxidants also. Thus catalase may play a role in scavenging luminal peroxide but its relative role compared to other antioxidant activities (Halliwell, 1996) in scavenging peroxide and other reactive oxygen species remains to be assessed.

The observation that endothelium can protect the underlying smooth muscle against luminally generated H_2O_2 may be clinically important in percutaneous transluminal coronary angioplasty and ischemia–reperfusion. It needs to be explored in order to know whether the damage caused in ischemic arteries by reactive oxygen species can be minimized by activating or inducing the endothelial catalase.

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References

Cosentino, F., Luscher, T.F., 1997. Endothelial function in coronary artery disease. Cardiologia 42, 1221–1227.

Darley-Usmar, V., Halliwell, B., 1996. Blood radicals: reactive nitrogen species, reactive oxygen species, transition metal ions, and the vascular system. Pharm. Res. 13, 649–662.

Elmoselhi, A.B., Blennerhassett, M., Samson, S.E., Grover, A.K., 1995. Properties of the sarcoplasmic reticulum Ca^{2+}-pump in coronary artery skinned smooth muscle. Mol. Cell Biochem. 151, 149–155.

Grover, A.K., Samson, S.E., 1989. Protection of Ca pump of coronary artery against inactivation by superoxide radical. Am. J. Physiol. 256, C666–C673.

Grover, A.K., Samson, S.E., 1997. Peroxide resistance of ER Ca^{2+} pump in endothelium: implications to coronary artery function. Am. J. Physiol. 273, C1250–C1258.

Grover, A.K., Samson, S.E., Fomin, V.P., 1992. Peroxide inactivates calcium pumps in pig coronary artery. Am. J. Physiol. 263, H537–H543.

Grover, A.K., Samson, S.E., Fomin, V.P., Werstiuk, E.S., 1995. Effects

of peroxide and superoxide on coronary artery: ANG II response and sarcoplasmic reticulum Ca^{2+} pump. Am. J. Physiol. 269, C546–C553.

Grover, A.K., Samson, S.E., Misquitta, C.M., 1997. Sarco(endo)plasmic reticulum Ca^{2+} pump isoform SERCA3 is more resistant than SERCA2b to peroxide. Am. J. Physiol. 273, C420–C425.

Grover, A.K., Samson, S.E., Misquitta, C.M., Elmoselhi, A.B., 1999. Effects of peroxide on contractility of artery rings of different sizes. Mol. Cell. Biochem. 194, 159–164.

Halliwell, B., 1996. Antioxidants in human health and disease. Annu. Rev. Nutr. 16, 33–50.

Luscher, T.F., Barton, M., 1997. Biology of the endothelium. Clin. Cardiol. 20, II10.

Shah, K.A., Samson, S.E., Grover, A.K., 1998. Effects of peroxide on endothelial nitric oxide synthase in coronary arteries. Mol. Cell Biochem. 183, 147–152.

Singal, P.K., Khaper, N., Palace, V., Kumar, D., 1998. The role of oxidative stress in the genesis of heart disease [editorial]. Cardiovasc. Res. 40, 426–432.

Van Breemen, C., Skarsgard, P., Laher, I., McManus, B., Wang, X., 1997. Endothelium–smooth muscle interactions in blood vessels. Clin. Exp. Pharmacol. Physiol. 24, 989–992.

Wu, K.D., Lee, W.S., Wey, J., Bungard, D., Lytton, J., 1995. Localization and quantification of endoplasmic reticulum Ca^{2+}-ATPase isoform transcripts. Am. J. Physiol. 269, C775–C784.