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HYDROGEN PEROXIDE ELICITS ACTIVATION OF BOVINE PULMONARY ARTERIAL SOLUBLE GUANYLATE CYCLASE BY A MECHANISM ASSOCIATED WITH ITS METABOLISM BY CATALASE

Michael S. Wolin and Theresa M. Burke

Department of Physiology, New York Medical College, Valhalla, New York 10595

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Guanylate cyclase activity in the soluble extract of bovine pulmonary arteries is activated by hydrogen peroxide generated by glucose oxidase only in the presence of catalase. This mechanism of guanylate cyclase activation is not blocked by scavengers for superoxide anion or hydroxyl radical, but is selectively inhibited by methylene blue, inactivation of catalase and ethanol. The time dependency of increases in guanylate cyclase activity in the presence of peroxides that are substrates for catalase are associated with the spectral detection of compound I, a species of catalase formed during the metabolism of peroxide. Thus, activation of soluble guanylate cyclase appears to be elicited by compound I of catalase or by a mediator generated by this species.

Studies on heme-containing soluble guanylate cyclase (EC4.6.1.2) indicate that enzyme activation is thought to be mediated through modification of the heme group, usually by binding free radicals, which presumably results in a change in the coordination of iron of the heme group to the enzyme (1). Currently, the physiological mechanism coupling receptor activation to soluble guanylate cyclase modulation appears to involve a calcium (2) and oxygen tension (3) dependent process which remains to be established.

Hydrogen peroxide was the first potential physiological agent to be associated with soluble guanylate cyclase activation (4). However, further study of the modulation of soluble guanylate cyclase activity by oxygen metabolites implicated hydroxyl radical (5), lipid peroxides (6) and superoxide anion (7) as the activators of soluble guanylate cyclase. In this study, we have reconstructed a mechanism of activation of soluble guanylate cyclase obtained from bovine pulmonary arterial smooth muscle by hydrogen peroxide, that appears to be mediated through its metabolism by catalase.

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MATERIALS AND METHODS

Materials - Deferoxamine mesylate (Desferal) as obtained from CIBA and ethylhydroperoxide from Ferrosan. Glucose oxidase from Aspergillis niger (4900 units/mg), catalase purified from bovine liver (type C-40, 11,000 units/mg), superoxide dismutase from bovine blood (2725 units/mg) and all other biochemicals were obtained from Sigma. Inorganic chemicals were analyzed reagent grade from Baker, [^{32}P]-GTP from New England Nuclear and [^{3}H]-cyclic GMP from Amersham.

Preparation of soluble guanylate cyclase - The main pulmonary artery obtained from calf lungs immediately after slaughter was dissected free of connective tissue, incubated in Krebs-bicarbonate buffer gassed with 95% O_2/ 5% CO_2 for two hours and immediately frozen with liquid nitrogen. The frozen vessels were pulverized in a specially constructed precision machined piston type tissue smasher at liquid nitrogen temperatures and homogenized in 25mM triethanolamine-HCl buffer (pH 7.4) plus 2mM glutathione (1 part tissue weight to 3 parts buffer volume) in an Eberbach 8580 semi-micro chamber on a Waring Blendor using 4-15 second bursts at full power. After filtering the white suspension through cheesecloth, the extract was centrifuged at 100,000 g for 1 hour at 4°C. The supernatant was stored in small aliquots at -60°C and protein was measured by the method of Bradford (8) using bovine serum albumin as a standard.

Determination of guanylate cyclase activity - Guanylate cyclase activity in the arterial supernatant was determined by a modification of procedures previously applied to bovine coronary arteries (9). Reaction mixtures (0.2ml final volume) contained 40mM triethanolamine-HCl (pH 7.4), 0.1mM GTP, approximately 700,000 cpm of [alpha-^{32}P] GTP, 1mM cyclic GMP, 40,000 cpm [^{3}H]-cyclic GMP (to monitor recovery, typically 40-60%), 2mM $MgCl_2$, 0.3mM 1-methyl-3-isobutylxanthine (MIX, to inhibit phosphodiesterase activity), 40ul of arterial extract (150-225μg tissue protein), 5mM glucose and test agents as indicated. Incubations were conducted at 37°C and terminated at 10 minutes or at the time indicated with a 200μl of ice cold 12mM EDTA and immediately sequentially chromatographed on Dowex-50(H^+)x4-400 and neutral alumina, prior to counting. The concentrations of all probes were selected to maximize guanylate cyclase activation or for inhibitors to not affect basal guanylate cyclase activity, unless indicated. (n=6-8).

Detection of compound I of catalase - This species was detected by the decrease in the absorbance at 405nm of the heme of catalase (10) under the conditions of guanylate cyclase assays, in the absence of GTP, cyclic GMP, $MgCl_2$ and MIX, on a Perkin Elmer Lambda 3b recording spectophotometer with thermostated cells. (n=4)

RESULTS AND DISCUSSION

Soluble guanylate cyclase activity is markedly enhanced by hydrogen peroxide generated by glucose oxidase in the presence of catalase, as indicated in Table 1. This mechanism of guanylate cyclase activation is not affected by scavenging superoxide anion with superoxide dismutase (SOD) or hydroxyl radical probes including mannitol, dimethylsulfoxide (DMSO) or deferoxamine. Inactivation of catalase with cyanimide (11) markedly inhibits guanylate cyclase activation that appears to be associated with peroxide metabolism by catalase. Ethanol, in addition to being an effective scavenger

TABLE 1

EFFECTS OF HYDROGEN PEROXIDE GENERATION AND OXYGEN METABOLITE SCAVENGERS ON GUANYLATE CYCLASE ACTIVITY

Condition	Guanylate Cyclase Activity (pmole cGMP/min./mg. protein)
No addition	4.1 ± 0.4
10mUnit/ml Glucose Oxidase (GO)	4.3 ± 0.9
GO + 1μM Catalase (CAT)	351 ± 46
GO + CAT + 10μg/ml SOD	320 ± 19
GO + CAT + 10mM Cyanimide	26 ± 15
GO + CAT + 50μM Methelene blue	8.7 ± 1.7
GO + CAT + 100mM Mannitol	367 ± 51
GO + CAT + 10mM DMSO	367 ± 35
GO + CAT + 1mM Deferoxamine	277 ± 41
GO + CAT + 100mM Ethanol	7.7 ± 1.0
0.1 mM Sodium Nitroprusside (SNP)	277 ± 38
SNP + 100 mM Ethanol	262 ± 29

of hydroxyl radical, is metabolized by the peroxidatic reaction of catalase resulting in a decrease in the steady-state level of the compound I species of catalase and the oxidation of peroxide to oxygen by Compound I (10). As indicated by the data in Table 1, ethanol selectively blocks guanylate cyclase activation by glucose oxidase/catalase and does not inhibit guanylate cyclase activation by nitroprusside.

The time dependence of guanylate cyclase activity in the presence of glucose oxidase generated peroxide, catalase and catalase plus several different peroxides are shown in Figure 1. Glucose oxidase alone displays a small rate of cyclic GMP formation that is approximately linear with time. Catalase alone elicits a larger rate of cyclic GMP formation that increases with time. Peroxides that are substrates for catalase, such as glucose oxidase derived hydrogen peroxide or ethylhydroperoxide (10), enhance the rate of cyclic GMP formation over that seen with catalase alone, in a manner that appears linear with time after the first minute of the assay. However, t-butylperoxide, which is not known to be a substrate for catalase, does not enhance the rate of cyclic GMP formation above the level of catalase alone.

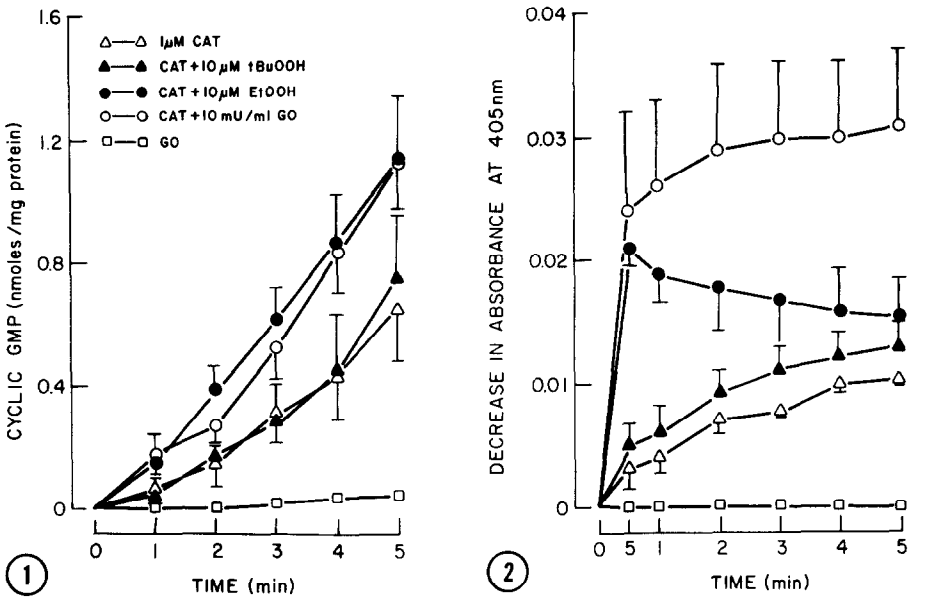

FIGURE 1. Effect of catalase and peroxides on the formation of cyclic GMP as a function of time. CAT = catalase, tBuOOH = t-butylhydroperoxide, EtOOH = ethylhydroperoxide, GO = glucose oxidase generation of hydrogen peroxide.

FIGURE 2. Time course for the formation of compound I of catalase under the conditions of the guanylate cyclase assays shown in Figure 1.

Thus, it appears that the metabolism of peroxide by catalase elicits the activation of guanylate cyclase and auto-oxidation of tissue or assay components may generate sufficient levels of peroxide to allow the expression of enzyme activation in the presence of catalase.

The decrease in absorbance at 405 nm of the heme of catalase was used to monitor the formation of compound I of catalase (10) as a function of time under the conditions of the guanylate cyclase assays shown in Figure 1. As shown in Figure 2, glucose oxidase in the absence of catalase does not cause a decrease in absorbance. In the presence of catalase and glucose oxidase or ethylhydroperoxide, a rapid and sustained decrease in absorbance is observed, suggesting the formation of compound I of catalase. In the absence of glucose oxidase generated peroxide, catalase displays a decrease in absorbance with time indicating that auto-oxidation of tissue or assay components is producing sufficient levels of peroxide to generate compound I of catalase. As might be anticipated, t-butylperoxide did not have a significant effect on the formation of compound I. Ethanol (100mM) completely abolishes all decreases

in absorbance and does not inhibit the generation of peroxide by glucose oxidase (not shown). Thus, the enhancement of guanylate cyclase activity (slope of data in Figure 1) appears to be associated with the formation of compound I of catalase. These results offer an explanation for the previously reported observation that purified heme-containing soluble guanylate cyclase can be activated by catalase under certain conditions (12), which are probably associated with the generation of peroxide from the auto-oxidation of dithiothreitol.

Since methylene blue (3) and ethanol (4) are two agents that have been reported to selectively antagonize the elevation of cellular levels of cyclic GMP to activation of muscarinic receptors, and these agents both selectively block the activation of soluble guanylate cyclase by the metabolism of peroxide by catalase (Table 1), the possibility exists that the mechanism described in this report might link receptor stimulation to soluble guanylate cyclase activation. We have recently found that relaxation of bovine pulmonary arteries by micromolar concentrations of hydrogen peroxide is associated with rises in tissue levels of cyclic GMP (15) and antagonized by methylene blue (15) or ethanol (16). Since hydrogen peroxide is a cellular metabolite whose formation might be controlled by oxygen tension (17), the mechanism of soluble guanylate cyclase activation described in this report could be involved in coupling the sensing of oxygen tension to the regulation of physiological processes such as vascular relaxation.

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