

Effect of Hydrogen Peroxide on Guinea Pig Nasal Mucosa Vasculature

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Received August 17, 2000 Accepted October 24, 2000

ABSTRACT—The effect of hydrogen peroxide (H_2O_2) on guinea pig nasal mucosa vasculature was studied by in vitro assay. H_2O_2 elicited relaxation of guinea pig nasal mucosa strips precontracted with phenylephrine in a concentration-dependent manner. The relaxant response to H_2O_2 was abolished in the presence of catalase. Preincubation of the strips with N^G -nitro-L-arginine methyl ester or methylene blue significantly attenuated the relaxant responses elicited by H_2O_2 . Fluorescence caused by DAF-2 DA, a fluorescence indicator for nitric oxide, was observed along the nasal mucosa vasculature in response to H_2O_2 . These results suggest that H_2O_2 induced relaxation of the guinea pig nasal mucosa vasculature and that this relaxation is mediated by the NO/cGMP pathway.

Keywords: Nasal mucosa vasculature, Hydrogen peroxide, Nitric oxide

Hydrogen peroxide (H_2O_2) produces vasodilatation in cerebral microcirculation (1) and elicits relaxation of isolated rabbit aorta (2) and bovine intrapulmonary artery (3). Vascular relaxation by H_2O_2 is believed to be activated by endothelial synthesis of nitric oxide (NO) (4) and cyclic guanosine 3',5'-monophosphate (cGMP) (3, 4). On the other hand, in rabbit lower urinary tract smooth muscle, H_2O_2 increased the relaxation and cGMP formation induced by NO and carbon oxide, although H_2O_2 alone failed to induce relaxation of the smooth muscle strips (5).

Ogasawara et al. (6) reported that the concentration of H_2O_2 in nasal secretions increased after nasal challenge with house dust and subsided gradually with an increase in peroxidase activity. However, the role of H_2O_2 in nasal diseases is not fully understood.

The purpose of this study was to investigate the effect of H_2O_2 on guinea pig nasal mucosa vasculature and its mechanism of action.

We used an in vitro assay method for detecting changes in muscle tension of the nasal mucosa vasculature (7). This study was approved by the Animal Welfare Committee of Hiroshima University School of Medicine. Male Hartley guinea pigs, weighing 300–500 g, were deeply anesthetized with pentobarbital sodium (35 mg/kg, i.p.) and killed by

exsanguination. The nasal mucosa was carefully dissected from the nasal septum with a sharp blade (8). Tissue strips of approximately 15 mm × 5 mm were vertically fixed by a hook in an organ bath containing 20 ml Krebs' bicarbonate solution aerated with a mixture of 95% O_2 + 5% CO_2 and maintained at 37°C. The composition of the solution was as follows: 119 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4 , 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$ and 11.1 mM glucose. The hook anchoring the upper end of the strip was connected to the lever of a force displacement transducer (model TB612T; Nihon Kohden, Tokyo). Resting tension was adjusted to an optimal tension of 0.5 g (9). Isometric contractions and relaxations were displayed on a pen-writing oscillograph (model SR 6211; Graphtec, Tokyo). Before the start of the experiment, the strips were left to equilibrate for 60 min. During this period, the bathing medium was replaced approximately every 15 min.

In order to obtain direct evidence for NO production in the nasal mucosa vasculature, we used 4,5-diaminofluorescein (DAF-2) as a fluorescent indicator for NO (10). The nasal mucosa strips (3 mm × 3 mm) were transferred to Krebs' bicarbonate solution and loaded with DAF-2 DA (10 μ M) for 1 h at 37°C in darkness. After DAF-2 DA loading, the specimens were rinsed with Krebs' bicarbonate solution and placed in an 8-well perfusion chamber (depth, 0.5 mm; diameter, 9 mm; volume, 3.5 μ l) (PC8R-0.5; Grace Bio-Labs Inc., Bend, OR, USA) filled with Krebs' bicar-

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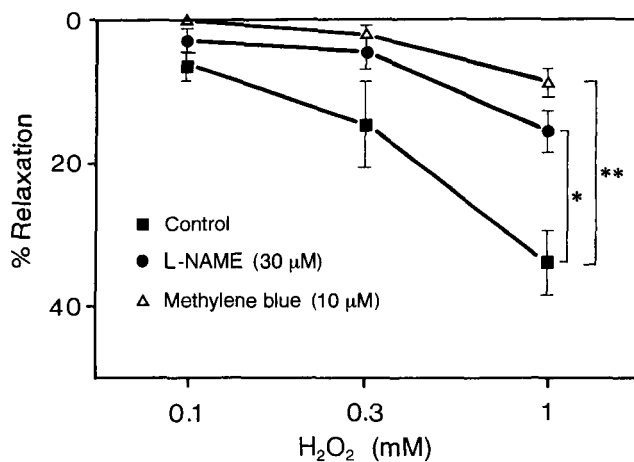


Fig. 1. Comparison of the effects of L-NAME and methylene blue on H₂O₂-induced relaxation of guinea pig nasal mucosa ($n=6$). H₂O₂ was administered after preparations were contracted with 30 μ M phenylephrine. % Relaxation represents percent change of the 30 μ M phenylephrine-induced contraction. The preincubation with L-NAME (30 μ M) or methylene blue (10 μ M) attenuated the relaxant responses to H₂O₂. Values represent means \pm S.E.M. of six experiments. * $P<0.05$, ** $P<0.01$ by Student's t -test.

bonate solution. For imaging, specimens were incubated at 37°C prior to use. Then, the specimens were loaded into 1 mM H₂O₂ and mounted on an inverted fluorescence microscope (Eclipse E600; Nikon, Tokyo) equipped with an excitation filter (465–495 nm), a dichroic mirror (505 nm) and an emission filter (515–555 nm). Fluorescence analogue images were obtained via an intensified color charge-coupled device (CCD) camera (C4742-95, Hamamatsu Photonics, Shizuoka).

The drugs used in the present experiment were phenylephrine, *N*^G-nitro-L-arginine methyl ester (L-NAME), methylene blue, catalase (Sigma, St. Louis, MO, USA), H₂O₂ (3% solution, Sankyo, Tokyo) and DAF-2 DA (Daiichi Pure Chemicals, Tokyo).

Cumulative application of H₂O₂ (0.1–1 mM) to the organ bath containing the guinea pig nasal mucosa strips precontracted with phenylephrine (30 μ M) caused relaxation of preparations in a concentration-dependent manner (Fig. 1). The relaxant responses were completely abolished in the presence of catalase (100 u/ml) in all 3 tissues (data not shown). L-NAME (30 μ M) or methylene blue (10 μ M) significantly reversed H₂O₂ (1 mM)-induced relaxation of nasal mucosa strips by approximately 30% ($n=3$) and 90% ($n=3$), respectively. Catalase (100 u/ml), L-NAME (30 μ M) and methylene blue (10 μ M) had no significant effect on the basal tone of preparations. The preincubation of strips for 20 min with L-NAME (30 μ M) or methylene blue (10 μ M) significantly ($P<0.05$ or $P<0.01$, respectively) attenuated the relaxation to H₂O₂ (Fig. 1).

A novel fluorescent indicator of NO, DAF-2DA, was hydrolyzed by cytosolic esterase to yield DAF-2 after penetration into the nasal mucosa tissue. DAF-2 DA is non-fluorescent, while DAF-2 is slightly fluorescent, so tissue loading could be confirmed by fluorescence measurement. In guinea pig nasal mucosa, the fluorescence was localized along the vasculature. Without stimulation (control state), there was a low level of fluorescence, but after adding H₂O₂ to the medium, the fluorescence intensity increased time-dependently and peaked within 40 min. Images of tissue at maximum fluorescence are shown in Fig. 2. In addition, we confirmed that the degree of fluorescence



Fig. 2. Fluorescence image of NO production in guinea pig nasal mucosa observed with DAF-2DA. The fluorescence was localized along the vasculature. This image was taken 40 min after loading with 1 mM H₂O₂, when the fluorescence peaked.

intensity induced by H_2O_2 concomitant with L-NAME (30 μM) was significantly attenuated (data not shown).

The vascular endothelium is sensitive to oxidative injury mediated by oxygen metabolites that are either released from inflammatory cells or produced by the endothelial cells themselves. Within the vascular system, the major source of H_2O_2 seems to be activated polymorphonuclear leukocytes (PMNs), since it has been shown that stimulation of PMNs leads to a rapid increase in the consumption of oxygen and the concomitant release of large amounts of oxygen-derived reactive species including O_2^- and H_2O_2 (11).

Ogasawara et al. (6) reported that the concentration of H_2O_2 in nasal secretions increased after nasal challenge with house dust and subsided gradually with the increase of peroxidase activity. The population of eosinophils and H_2O_2 generation, morphologically detected on the plasma membrane of eosinophils in nasal secretion, increased with eosinophil chemotactic activity after nasal challenge. H_2O_2 generation by eosinophils appears to be an important event in tissue injury and augmentation of allergic reaction (6), but the functional role of H_2O_2 in nasal diseases is not fully understood. We hypothesize that H_2O_2 elicits the nasal mucosa vasodilator response, thereby affecting the modulation of blood flow and the swelling of nasal mucosa.

In the present study, we used H_2O_2 at 0.1–1 mM because H_2O_2 at millimolar concentrations has been used extensively as a model for experimental oxidative stress (4). H_2O_2 elicited the concentration-dependent relaxation in nasal mucosa. This is the first demonstration of an H_2O_2 -induced relaxant effect on the nasal mucosa.

It has been reported that H_2O_2 elicits relaxation of isolated rabbit aorta (2) and bovine intrapulmonary artery (3). The mechanism responsible for vascular relaxation by H_2O_2 is said to be activated by endothelial synthesis of NO (4) and elevation of cGMP level in smooth muscle cells (3, 4), but the process of increased cGMP formation by H_2O_2 is not fully understood. H_2O_2 alone had no effect on cGMP formation in the rabbit lower urinary tract smooth muscle, indicating that whatever the mechanism of action, a ligand for guanylate cyclase must be present (5). H_2O_2 -induced concentration-dependent relaxation in the bovine intrapulmonary artery via the stimulation of soluble guanylate cyclase has been reported to be independent of the endothelium (3). On the other hand, Zembowicz et al. (4) reported that H_2O_2 -induced relaxation in the rabbit aorta was comprised of both endothelium-dependent and -independent components and that H_2O_2 elicited concentration-dependent relaxation in the presence of intact endothelium 2–3-fold larger than in its absence in the rabbit aorta. In addition, L-NAME blocked the endothelium-dependent component of the relaxant action of H_2O_2 . Therefore, the endothelium-dependent component of the relaxant action

of the tissue is due to enhanced endothelial synthesis of NO (4). One plausible mechanism of NO production by H_2O_2 is explained by the fact that intracellular calcium concentrations ($[\text{Ca}^{2+}]_i$) in endothelial cells is increased by H_2O_2 . H_2O_2 can raise $[\text{Ca}^{2+}]_i$ (12), which is required for constitutive NO synthase (cNOS) and thereby stimulates formation of NO and L-citrulline from L-arginine. NO may antagonize the vasoconstrictor effects of other PMNs-derived mediators such as O_2^- (13), thromboxane A_2 (14) and peptido-leukotrienes (15). Thus, the augmentation of the production of NO by H_2O_2 may serve as a negative feedback mechanism by which the activation of PMNs adhering to the endothelium is modulated by endothelium-derived NO (4).

Kojima et al. (10) reported that the fluorescence intensity in the CA1 region of the hippocampus was augmented after stimulation with NMDA and concluded that this was direct evidence of NO production there. The present results of the imaging study in guinea pig nasal mucosa implied that the increase in fluorescence intensity was due to formation of DAF2-T and NO (10). Thus, production of NO in the nasal mucosa after stimulation with H_2O_2 was suggested. These results strongly suggest that NO production along the vasculature is augmented by H_2O_2 . We conclude that the relaxant mechanism elicited by H_2O_2 in the guinea pig nasal mucosa is mediated by the NO/cGMP pathway.

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