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# HYDROGEN PEROXIDE : AN ENDOGENOUS SMOOTH MUSCLE CELL HYPERPOLARIZING FACTOR[1]

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Hydrogen peroxide can be released by different cells such as the nerves, the endothelial or phagocytotic white blood cells which can all interact with vascular smooth muscles. We show that hydrogen peroxide hyperpolarizes and relaxes pig coronary artery smooth muscle cells. The possibility that the endothelium derived hyperpolarizing factor released by the endothelium in response to bradykinin and substance P being hydrogen peroxide was tested using catalase, an enzyme which hydrolyses hydrogen peroxide. We find that this particular endothelial hyperpolaririzing factor and hydrogen peroxide are two distinct molecules. 

The local control of vascular smooth muscle is carried out by factors released from nerve varicosities, the endothelium, and in inflamation by phagocytotics white blood cells such as macrophage or neutrophils or the smooth muscle cells themselves. All these cells have been shown to produce oxygen derived free radicals superoxide anions or their derivative hydrogen peroxide or hydroxyl radicals (1,2,3,4,5). Using canine coronary arteries Needleman et al. (6) and Rubanyi and Vanhoutte (7) demonstrated that hydrogen peroxide and not superoxide anion or hydroxyl radical relaxes these vessels.

Some agents relaxe smooth muscle cells by hyperpolarizing them (8). The change in membrane potential probably closes voltage dependent $Ca^{++}$ channels leading to a decrease in cytosolic

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$Ca^{++}$ which results in relaxation. In this paper we address the question of the effect of hydrogen peroxide on membrane potential of pig coronary artery smooth muscle cells. We report here that hydrogen peroxide hyperpolarizes the vascular smooth muscles causing concomitantly its relaxation.

In addition to nitric oxide (9), the endothelial cells release a as yet uncharacterized endothelium-dependent hyperpolarizing factor (EDHF) which hyperpolarizes and relaxes the arterial smooth muscles (10). In the context of the observations presented above, hydrogen peroxide seems to be a possible candidate for EDHF since it relaxes and hyperpolarizes the smooth muscles and is produced by the endothelium. Therefore we conducted experiments to find whether the EDHF released by the endothelium in response to substance P and bradykinin is hydrogen peroxide.

## METHODS

The method is described in detail elsewere (11). Briefly: mechanical tension or force and transmembrane potential were measured simultaneously.on a strip ( 1 X 5 mm) of the anterior descending branches of pig coronary arteries. In some experiments the endothelium was removed using a cotton tip. The absence of relaxation of the strip in response to substance P was a control of the deendothelialisation.

### Drugs used in this study

Acetylcholine chloride, prostaglandin $F_{2\alpha}$, deferoxamine mesilate and catalase from bovine liver (50 U/mg protein) were obtained from Sigma, ferrous gluconate, D- mannitol and hydrogen peroxide 30 % from Fluka, Switzerland, synthetic substance P and bradykinin were obtained from Bachem Feinchemikalen, Switzerland.

### Data analysis

Data were calculated as the mean ± standard error of the mean (SEM). The student's *t* test was used to compare results, $p < 0,05$ deemed to be significant.

$IC_{50}$ was calculated for each concentration-response curve by linear interpolation between two points on either side of the 50 % of the maximal response (100 %) and by reading the corresponding concentration on the logarithmic scale. These readings were used to calculate the mean ± SEM.

## RESULTS AND DISCUSSION

### Effect of hydrogen peroxide

A concentration of 10 µM hydrogen peroxide has no effect on a deendothelialized strip contracted by 10 µM prostaglandin $F_{2\alpha}$, this contraction is inhibited by 59 ± 12 % (n=4) and 104 ± 4 % (n=4)

by 100 μM and 1 mM hydrogen peroxide respectively (Fig.1). Figure 1 (lower panel), shows that the relaxing effect of hydrogen peroxide is reversible and that it could be repeated. When the deendothelialized strip is not contracted hydrogen peroxide at concentration from 10 μM to 10 mM causes no change in isometric

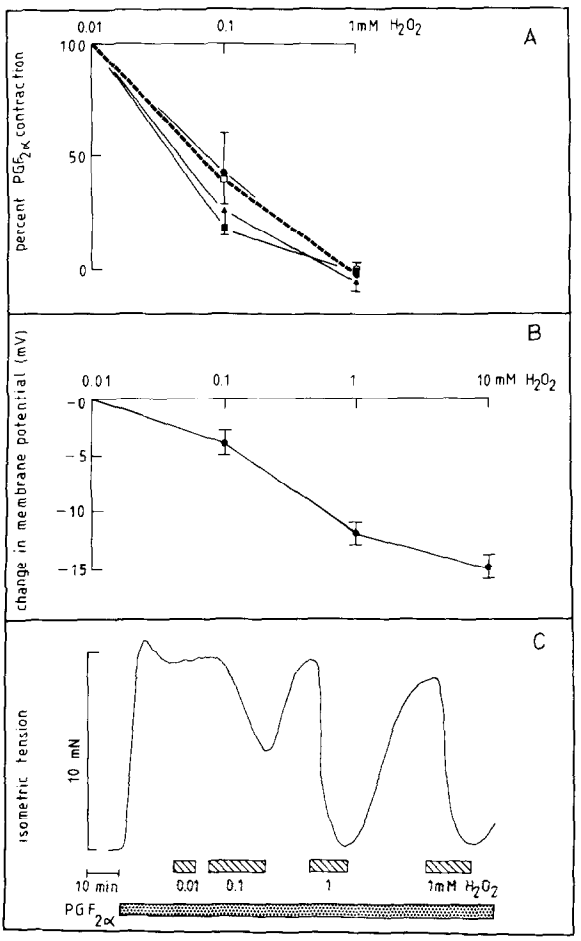


Fig 1

Panel A. Thick interrupted line □: effect of different concentrations of hydrogen peroxide on the isometric tension (in percent of supramaximal $PGF_{2\alpha}$ contraction) of a pig coronary artery strip without endothelium contracted by 10 μM $PGF_{2\alpha}$. ●: in the presence of 80 mM mannitol. ▲: in the presence of 1 mM ferrous ions. ■: in the presence of 1 mM deferoxamine.

Panel B. Effect of different concentrations of hydrogen peroxide on the smooth muscle cell membrane potential.

Panel C. Effect of different concentrations of hydrogen peroxide on the isometric tension ( in millinewton: mM) of a pig coronary artery strip without endothelium contracted by 10 μM $PGF_{2\alpha}$.

tension. Hydrogen peroxide at 10 mM causes a weak contraction (4 % ± 0,2 ,n=8, of a phasic supramaximal ACh contraction) when the strip is tonically contracted by acetylcholine.

On a strip without endothelium, hydrogen peroxide at 100 µM, 1 mM and 10 mM concentrations hyperpolarize the smooth muscle cells membrane potential by 4 ± 0.6 mV (n=6), 12 ± 1 mV (n=4) and 15 ± 1 mV (n=5) respectively (Fig.2). In these series of experiment the membrane potential of the recorded cells was respectively -48 ± 1 (n=6), - 50 ± 2 (n=4) and -45 ± 2 mV (n=5) before the application of hydrogen peroxide. The observation that hydrogen peroxide hyperpolarizes pig coronary artery smooth muscle cells furnishes a possible explanation of its relaxing effect (8).

The enzyme catalase scavenges hydrogen peroxide to generate water and oxygen (7,12). We verified that this enzyme inhibits the effect of hydrogen peroxide. A concentration of 4,000 U/ml catalase suppresses the inhibition of the prostaglandin $F2\alpha$ contraction caused by hydrogen peroxide.

Hydroxyl radicals

Hydrogen peroxide could interact in situ with superoxide anion radical to form hydroxyl radical (7,12). Therefore we tested

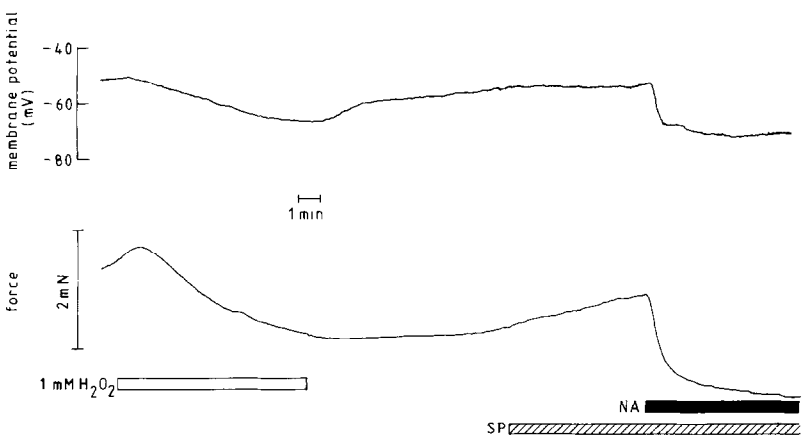


Fig 2

Simultaneous recording of the membrane potential (in millivolts : mV) of a smooth muscle cell and of the isometric tension (in millinewtons: mN) of the strip.
The strip is contracted by a continuous perifusion of 10 µM ACh (ACh have no effect on the membrane potential of these cells(16)). Effect of 1 mM hydrogen peroxide ($H_2O_2$). 175 nM substance P (SP) is applied to test for the absence of the endothelium since in the presence of endothelium SP relaxes and hyperpolarizes these cells (14). By comparison the effect of 10 µM noradrenaline is also shown.

whether these radical would be responsible for the effect of hydrogen peroxide. To do this we used mannitol, ferrous ions and deferoxamine. The rational of these experiments were as follows: Mannitol inhibits the spontaneous destruction of the hydroxyl radical. Therefore if this radical is the active molecule, mannitol would shift the concentration-response curve of hydrogen peroxide to the left. The reaction of Haber-Weiss and Fenton which form the hydroxyl radical is facilated by ferrous ions and inhibited by deferoxamine which scavenges the catalytic iron (12). Therefore in the presence of ferrous ion, the concentration-response curve of hydrogen peroxide would also be displaced to the left. However in the presence of deferoxamine, the concentration-response curve would be displaced to the right if hydroxyl radical is the active relaxant. The concentration-response curve of hydrogen peroxide using deendothelialized strip (contracted by constant perifusion with 10 μM prostaglandin $F_{2\alpha}$) was not significantly changed by the presence of 80 mM mannitol, 1 mM ferrous gluconate or 1 mM deferoxamine (Fig.1). When 80 mM mannitol was added to the Krebs solution, 10 mM hydrogen peroxide hyperpolarization came to a value of 19 ± 3 mV (n=4). In this serie of experiments the membrane potential of the smooth muscle cells was -46 ± 2 mV (n=4), but in that case, the solution is hyperosmotic. In order to avoid an effect of the hyperosmolarity, (in another set of experiment) 40 mM NaCl were substracted to the Krebs solution to compensate for the osmolarity of added mannitol. In this series of experiments the membrane potential of the smooth muscle cells was -45,5 ± 3 mV (n=4). The perifusion with this solution hyperpolarizes in itself the smooth muscles by 8,5 mV ± 1,5 (n=4) which thus reach a potential of -54 ± 2 mV (n=4) and the hydrogen peroxide futher hyperpolarizes these cells by 16 ± 1,7 (n=4). From these results we conclude that hydrogen peroxide and not hydroxyl radical hyperpolarizes and relaxes the pig coronary arteries smooth muscle, this conclusion is compatible with the observation that coronary arteries of the dog and rabbit aorta are also relaxed by hydrogen peroxide (6,7,13), although in these studies the membrane potentials were not measured.

Is hydrogen peroxide the EDHF released by the endothelium?

The observation that hydrogen peroxide relaxes and hyperpolarizes the smooth muscles independently of the endothelium by a direct effect on the smooth muscles makes it a candidate for the unidentified EDHF released by the endothelium in response to bradykinin or substance P. Particularly, since

bradykinin induces superoxide anion release from human endothelial cells (5). If this hypothesis is correct, catalase should inhibit the endothelium-dependent hyperpolarizations caused by both kinins since the enzyme destroys the hydrogen peroxide. The continuous perifusion of coronary strips with 4,000 U/ml catalase does not significantly change the effective concentration giving 50 % of maximal relaxation ($EC_{50}$) of both substance P and bradykinin. $EC_{50}$ was respectively 0.3 ± 0.03 nM (n=5) and 3.5 ± 0.16 nM (n=4) in the presence and respectively 0.38 nM and 2.6 nM in absence of catalase (11,14). In the same way, catalase does not change the endothelium-dependent hyperpolarization caused by both kinins.(175 nM substance P and 225 nM bradykinin) The substance P hyperpolarization was 16 ±2 mV (n=4) in presence of catalase. During these experiments the membrane potential of the cells was -49 ± 2 mV (n=4). By comparison, the hyperpolarization caused by substance P in the absence of catalase was 12 to 17 mV (14, 15). Likewise, the bradykinin hyperpolarization was 16 ± 2 mV (n=4) in the presence of catalase. This value must be compared to the hyperpolarization caused by bradykinin in the absence of the enzyme which was from 11 to 17 mv (11). During this set of experiments the membrane potential of the cells was 49 ± 1 mV (n=4). These results indicate that the endothelium derived hyperpolarizing factor released in response to substance P and bradykinin is not hydrogen peroxide. It could be argued that catalase cannot reach the location where this hypothetic factor is released. However catalase was already used with success in a similar experimental design by Katusic et al. (12). Another fact speaks against the idea that hydrogen peroxide is the EDHF. The speed of hyperpolarization of the smooth muscles cells is faster for the kinins hyperpolarization than for the hydrogen peroxide hyperpolarization. The time to reach 50 % of the maximal hydrogen peroxide hyperpolarization is 47 ± 3 sec (n=4), whereas it is less than 10 sec for the bradykinin as well the substance P hyperpolarization (11,14).

Consequently the inability of catalase to inhibit the endothelium-dependent hyperpolarizations caused by substance P and bradykinin plus the difference in the temporal evolution between the kinins and the hydrogen peroxide hyperpolarizations prove that hydrogen peroxide is not the EDHF released by the pig coronary endothelium in response to kinins, despite it is able to relaxe and hyperpolarize the smooth muscle cells.

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