Free Rad. Res. Comms., Vol. 7, No. 3-6, pp. 265-274
Reprints available directly from the publisher
Photocopying permitted by license only

PHYSIOLOGICAL TARGETS OF SUPEROXIDE ANION AND HYDROGEN PEROXIDE IN REPERFUSION INJURY

V. ULLRICH,‡ B. BRÜNE, G. HECKER, K.-U. SCHMIDT, A. MÜLSCH§ and R. BUSSE§

Faculty of Biology, University of Konstanz, 7750 Konstanz, F.R.G. §Department of Applied Physiology, University of Freiburg, Hermann-Herder-Str. 7, 7800 Freiburg i. Br., Fed. Rep. Germany

Current dogma associates reperfusion injury with the introduction of reactive oxygen species (ROS) into the ischemic tissue. The sources of ROS under discussion are xanthine oxidase in the endothelium of small vessels and/or invaded polymorphonuclear leukocytes (PMN). The beneficial effects of both superoxide dismutase and catalase suggest an involvement of superoxide anions and hydrogen peroxide in this pathophysiological process, without describing the targets of their action.

In our work we demonstrate that these two ROS effectively interact with two enzymes. Superoxide anions inhibit soluble guanylate cyclase. Its product, cGMP, is considedred to antagonize platelet activation and to cause smooth muscle relaxation. Thus O_2^- can intensify platelet aggregability and small vessel occlusion. Similar effects are elicited by H_2O_2, which shifts the dose response curve of several agonists towards smaller concentrations by activating cyclooxygenase. This enzyme provides the substrate for thromboxane synthase which generates TxA_2, the most potent physiologically occurring platelet aggregating and smooth muscle contacting agonist.

These results lead us to the suggestion that the influence of the oxidative burst of PMN in the phenomenon of reperfusion injury should be reconsidered.

KEY WORDS: Reperfusion injury, platelet aggregation, superoxide anions, guanylate cyclase, hydrogen peroxide, cyclooxygenase.

ABBREVIATIONS USED: cGMP, cyclic 3',5'-guanosine monophosphate; CO, carbonmonoxide; EDRF, endothelium derived relaxing factor; GTP, guanosine 5'-triphosphate; EDTA, ethyleneglycol-bis(β-aminoethylether)-N,N,N',N'-tetraacetic acid; HHT, 12-(S) hydroxy-5,8,10-heptadecatrienoic-acid; 12-(S)HETE, 12-hydroxy-5,8,10,14-eicosa-trienoic acid; p.p.GC, partially purified guanylate cyclase; s GC, soluble guanylate cyclase; SU, supernatant; SOD, superoxide dismutase [EC 1.15.1.1]; TxA_2, thromboxane A_2; XO, xanthine oxidase [EC 1.2.3.2].

INTRODUCTION

The severe tissue damage by post-ischemic reperfusion is well documented but the mechanistic explanations for its generation are not satisfactory. After occlusion of myocardial vessels, great efforts are undertaken to reperfuse the infarcted area since mortality is directly related to infarct size. This reperfusion, however, is accompanied by an extension of the tissue damage as compared to that caused by permanent ischemia alone.

Current knowledge associates this "reperfusion injury" to the formation of reactive oxygen species (ROS).[1] Possible sources of ROS include mitochondria,[2,3] xanthine

‡To whom reprint requests should be sent.

oxidase,[4-6] and invaded neutrophils.[7] Though the existence of xanthine oxidase in human myocardial tissue has been questioned,[8] the protective effects of allopurinol, a selective XO-inhibitor, during reperfusion experiments favour the thesis of this enzyme to be a potent source of $O_2^{\cdot-}$ during myocardial ischemia.[9] Meanwhile, it is believed that xanthine oxidase is located rather in the endothelium of microvessels than in large vessels or myocytes.[10]

Reperfusion of the infarcted myocardium is acompanied by an increased adherence of neutrophils to the vascular endothelium. It has been speculated that this increased adhesiveness after ischemia is mediated by the expression of a newly synthesized cell surface structure on endothelial cells which has been called endothelial leukocyte adhesion molecule-1(ELAM-1).[11]

Besides the possibility that PMN become activated by the change in oxygen tension itself,[12] additional chemoattractants and activators like 12-HETE[13] and complement factors (C3b; C5a)[14,15] can be released by the endothelium. Maroko *et al.*[16] reported that inhibition of complement system activation resulted in a significant reduction in infarct size.

After activation, PMN release several mediators like lyososomal enzymes, superoxide anions and metabolites of the arachidonic acid cascade.[13] Activated PMN aggregate in microvessels and lead to the so-called "no flow phenomenon".[17]

Romsen and coworkers[18] depleted dogs of circulating neutrophils and rendered the heart ischemic followed by a reperfusion period. The resulting infarct size was reduced by over 40% as compared to non-neutropenic dogs. Thus, neutrophils are directly involved in the enhancement of myocardial damage during reperfusion. These results were confirmed by several other groups.[19] Since inhibition of lysosomal protease activity did not influence infarct size[20] whereas infusion of superoxide dismutase and catalase provided distinct protection during reperfusion[1] oxygen metabolites of PMN are most likely the mediators of toxicity.

Although the generation of hydroxyl radicals by the modified Haber–Weiss reaction is the genrally accepted pathway of ROS formation,[21,22] we also considered a direct action of $O_2^{\cdot-}$ and H_2O_2, specially on platelet aggregation. Likewise, hydrogen peroxide has been reported to support platelet aggregation.[23-25] $O_2^{\cdot-}$ also leads to platelet activation in the presence of catalase, thus suggesting that its action is independent of H_2O_2 formation.[26] From previous work on carbon monoxide-induced inhibition of platelet aggregation we noticed the crucial role of cGMP levels in controlling platelet activity.[27] Nitric oxide (NO) which recently has been identified as EDRF[28] is also a positive effector of sGC and being a radical it could effectively be trapped by $O_2^{\cdot-}$. Even a direct interaction with the regulatory subunit of sGC would be possible.[29]

To test this hypothesis we partially purified sGC from human platelets and measured the effects of $O_2^{\cdot-}$ on the CO-activated enzyme.

Our results favour a potent inhibitory action of $O_2^{\cdot-}$ on cGMP formation in platelets and possibly other tissues. H_2O_2 stimulates platelet arachidonate metabolism by H_2O_2 which seems to involve cyclo-oxygenase activation.

MATERIALS AND METHODS

Materials

Apyrase, grade I, from potato, heparin grade I, from porcine intestinal mucosa,

hydrogen peroxide, aspirin, indomethacin, xanthine, xanthine oxidase and superoxide dismutase were purchased from Sigma Chemie (Deisenhofen, FRG). CO and N_2 gas were from Messer-Griesheim, FRG. ^{14}C-arachidonic acid and ^{32}P-GTP were from DuPont (Dreieich, FRG). PGI_2 was kindly provided by Prof. Stock, Schering AG (Bergkamen, Berlin, FRG) and BM 13.177 was a kind gift of Prof. H. Patscheke, (Mannheim, FRG). The Zetaprep DEAE cartridges were kindly supplied by CUNO-Europe (Mainz, FRG). All other reagents and solvents were obtained in p.a. quality or in the highest quality available from Aldrich Chemie (Steinheim, FRG), E. Merck, (Darmstadt, FRG) or Riedel-de-Haen (Seelze, FRG).

Preparation of human platelets

Platelets were prepared from blood of healthy human volunteers who had not taken any drugs during the last fortnight. The blood was anticoagulated with 3.8% sodium citrate (1/10, v/v) and centrifuged for 20 min at 200 × g. Washed human platelets were prepared essentially according to the method published by Siess *et al*[30] modified by further addition of PGI_2 (1 μM).

Measurement of platelet aggregation

Aggregation of platelets in HEPES-buffer in response to agonists was monitored continuously with an ELVI 840 aggregometer following the method described by Born.[31]

Measurement of 20:4 metabolism by TLC

Washed human platelets were stimulated by ^{14}C-20:4 and the reaction was followed for 3 min. Thereafter the incubates (1 ml) were stopped by methanol (1 ml) and acidified to pH 3 with 0.6% formic acid (0.6 ml). The samples were extracted by chloroform/methanol (2.1 ml; 3:1). After centrifugation (3 min; 725 × g) the chloroform phase was removed and evaporated to dryness under nitrogen. The extracts were dissolved i n75 μl chloroform and quantitatively applied to TLC-plates precoated with silica-gel 60 (Merck, Darmstadt, FRG). To determine 20:4 metabolites the following solvent system was applied: trimethylpentane/ethylacetate/HAc/H_2O/(50:90:20:100; by vol., top phase). The chromatograms were counted on a thinlayer linear analyzer (Isomess, Straubenhardt, FRG).

Preparation of a platelet 10,000 × g supernatants

The activity of guanylate cyclase was determined in a freshly prepared 10,000 × g supernatant. Washed human platelets were resuspended in 20 mM triethanolamine buffer pH 7.4 containing 0.2 mM EDTA and 0.2 mM benzamidine, gassed with nitrogen for 5–10 min and then sonicated in an ice-water bath with a Branson sonifier (model B30, power setting at 10, 3/4-inch probe) for 10 s. The procedure was repeated 8 to 10 times with 1 min intervals before centrifugation (10 min, 10,000 × g).

Purification of soluble guanylate cyclase from human platelets

The soluble enzyme of human platelets was purified following basically the method

described by Gerzer *et al.*[32] Briefly, the 100,000 × g supernatant of human platelets was subjected to anion exchange chromatography on Zetaprep 3200 DEAE cartridges (Pharmacia-LKB, Freiburg, FRG), followed by ammoniumsulfate precipitation, dialysis against low ionic strength buffer and subsequent affinity chromatography on Blue Sepharose. After concentration the fractions were loaded on native polyacrylamide gel for preparative electrophoresis. After continuous elutions the fractions containing guanylate cyclase were concentrated and stored in 50% glycerol at − 70°C under nitrogen.

Determination of guanylate cyclase activity

Guanylate cyclase activity was determined in a total volume of 0.2 ml with 30 mM triethanolamine/HCl buffer pH 7.4 supplemented with 3 mM $MgCl_2$, 0.1 mM IBMX, 3 mM glutathione, 5 mM creatine phosphate, 5 U creatine kinase, 0.05 mM xanthine, platelet SU as indicated and in the experiments with partially purified enzyme also 0.1 mg/ml bovine gamma-globulin. The reaction was started by adding 0.1 mM guanosine triphosphate, 200,000–400,000 cpm [α-^{32}P]GTP and 0.1 mM cyclic guanosine monophosphate. After incubation at 37°C the reaction was stopped by adding 0.45 ml of 120 mM zinc acetate followed by 0.6 ml of 120 mM sodium carbonate. ^{32}P cGMP was isolated by chromatography on acid aluminium oxide (recovery 50%).

Activation by CO was obtained by adding CO-saturated water and in the case of 10,000 × g SU, the protein was also gently bubbled with 95% CO/5%O_2 for 5 min. Controls were teated with 95% N_2/5%O_2 and/or with N_2-saturated water. Commercially available preparations of xanthine oxidase were centrifuged at 10,000 × g for 10 min and resuspended in 20 mM triethanolamine buffer pH 7.4 before use in order to remove ammoniumsulfate.

Statistics

Results are expressed as mean values of n determinations ± SEM of individual experiments.

RESULTS

Using our purification procedure we achieved an about 1000-fold enrichment of guanylate cyclase activity. The presence of a hemoprotein was supported by the finding of a spectral shift with the active fraction showing absorption bands at 415 and 575 nm. Carbon monoxide shifted the Soret band to 420 nm (Figure 1).

As shown in Table 1 the enzymatic activity of this preparation as well as of the 10,000 × g supernatant of platelets or in the partially purified enzyme preparation was significantly stimulated by CO. Control experiments using N_2 instead of CO indicate that carbon monoxide does not act via replacing oxygen in the sample. The specificity of its action was further supported by the photo-reversibility of the activating effect (data not shown). The whole spectral region from a 250 W quartz lamp was used and about 50% of the CO-dependent stimulation was suppressed. Without the addition of CO light did not change the enzyme activity. Thus the view is supported that it is the ligation of CO to the ferrous heme which causes activation of GC.

It is obvious that CO is not a physiological regulator of GC activity and, therefore,

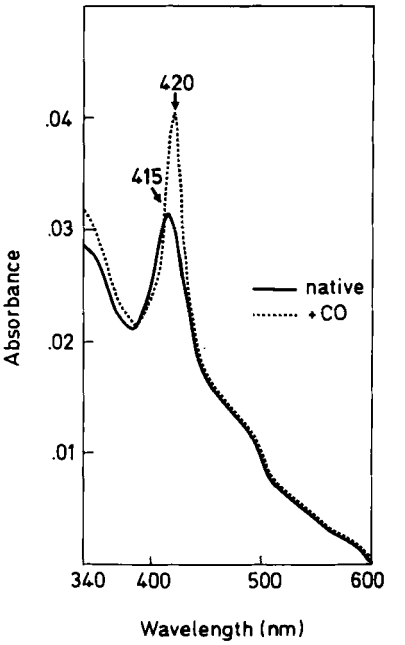

FIGURE 1 The assay was performed with 460 μg platelet protein at room temperature. The solid line demonstrates the absorption spectrum of native sGC (10,000 × gSU) and the dotted line represents the shift of the Soret band in the presence of 100 μM CO in the solution.

TABLE I

Activation of sGC in platelet 10,000 × g supernatant. The assay was performed with 3 mM Mg^{2+}, 100 μg 10,000 × g platelet protein (10,000 × gSU). for 10 min at 37°C. N_2-saturated water was added to the same amount as CO-saturated solution.

Addition	10,000 × gSU [cGMP, pmol × min^{-1} × mg^{-1}]	n
None	5.5 ± 2.6	3
SOD (1000 U/ml)	9.3 ± 2.3	3
CO (500 μM)	74.1 ± 18.5	3
CO + SOD	190.7 ± 19.1	5
SNP (100 μM)	798.6 ± 314.4	5

we tested the possibility that in our system CO was preventing an inactivation of the enzyme by a yet unknown agent. Among the chemicals described as activators or inhibitors of the enzyme, is superoxide which could inhibit the enzyme.[29] Indeed, platelets made insensitive by CO towards aggregating agonists like arachidonate, collagen or thrombin could be activated in the presence of an $O_2^{\cdot-}$ generating system (Brüne, unpublished results). Its action could be traced back to reduced cGMP levels when a platelet homogenate was stimulated with CO and then increasing xanthine oxidase concentrations were added to a xanthine supplemented incubation mixture

TABLE II

Effects of superoxide anions on the activity of partially purified sGC. The assay was performed with 3 mM Mg^{2+}, and 2.2 μg protein of the partially purified enzyme (p.p. sGC) per 10 min at 37°C. Each sample was supplemented with 0.1 mM xanthine. The data shown here are a representative experiment out of five different preparations. The 100% value amounts to 6300 pmol cGMP $\times$ min^{-1} $\times$ mg^{-1}. 13.4 μg xanthine oxidase generates 0.33 nmol $O_2^{\cdot-}$/ml $\times$ min in the presence of 0.1 mM xanthine.

Addition	p.p. GC-activity [% of control]
none	100
none + XO	75
none + XO (2.7 μg/0.35 mU)	48
none + XO (3.4 μg/0.44 mU)	31
none + XO (6.7 μg/0.87 mU)	25
none + XO (13.4 μg/1.74 mU)	13

(data not shown). Similar results were obtained by using the 1000-fold enriched enzyme purification (Table 2).

The inhibition by $O_2^{\cdot-}$ turned out to be reversible which is in favour of the hypothesis that superoxide could act as a physiological regulator of cellular GC activity.

SOD, even at high concentrations, could not significantly enhance the basal activity which at first sight disproved the hypothesis that CO was antagonizing the inhibitory action of endogenous $O_2^{\cdot-}$. However, when CO was added in order to activate the enzyme, a large stimulating effect of SOD was seen (Table 1). Thus superoxide anion is able to regulate the cellular concentrations of an important second messenger system. This occurs by a direct interaction with an enzyme since in this system we can rule out scavenging effects for NO. Furthermore, no additional factors like free ions, as postulated in the modified Haber–Weiss cycle[33] are required for this inhibitory action.

The protective effects of catalase during reperfusion injury support the assumptions of an involvement of H_2O_2 during this pathophysiological process.

We were therefore interested in finding additional functions of hydrogen peroxide in "reperfusion injury". A possible target could be platelets. We concentrated on the effect of H_2O_2 on stimulation of platelets by subthreshold concentrations of agonists (adenosinediphosphate; epinephrine; arachidonic acid; thrombin; collagen, Ca-ionophore and phorbol-myristate acetate;). The chosen stimulus concentrations did not induce platelet aggregation, however, simultaneous addition of hydrogen peroxide to each of the agonists was followed by complete platelet stimulation characterized by aggregation, secretion and arachidonate metabolism.

Figure 2 shows this effect for arachidonic acid (20:4). The significant shift of the dose response curve to the left (Figure 2) by simultaneous addition of H_2O_2 was accompanied by an increased production of arachidonate metabolites, i.e. TxB_2, HHT and 12-HETE (Table 3). Formation of TxB_2 of HHT increased by 200–300% and 12-HETE by about 50–75%. Essentially similar results were obtained with all agonists tested. The proaggregatory effects of H_2O_2 could be antagonized by the cyclooxygenase inhibitors aspirin or indomethancin as well as the thromboxane/endoperoxide receptor antagonist BM 13,177.

No further stimulating capacities of H_2O_2 could be detected when the agonist concentrations were significantly raised above subthreshold levels.

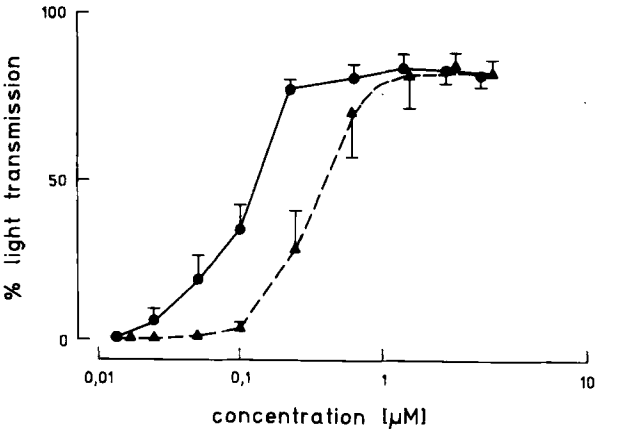

FIGURE 2 After 2 min of preincubation at 37°C and 800 rpm washed human platelets (2–4 × 10^8/ml) were stimulated for 3 min by increasing concentrations of 20:4 [▲]. Concomitant addition of H_2O_2 (10 μM) [●] significantly shifted the dose response curve to the left. The values are means ± S.E.M. of five different preparations. The cuvette containing buffer is referred to as 100% light transmission.

TABLE III

Effects of H_2O_2 on arachidonic acid metabolism in platelets. Washed human platelets (2–4 × 10^8/ml) were preincubated at 37°C and 800 rpm for 2 min followed by stimulation with ^{14}C-arachidonic acid. Simultaneously H_2O_2 or buffer were added. 3 min later the samples were stopped and further treated as described under Materials and Methods. The values given here are the mean ± SEM of five different experiments.

Agonist		TXB_2 [%]	HHT [%]	12-HETE [%]
20:4	(0.1 μM)	6.5 ± 1.9	6.4 ± 1.5	23.8 ± 2.1
20:4 + H_2O_2	(1 μM)	18.1 ± 2.8	19.3 ± 3.7	28.4 ± 3.8
20:4 + H_2O_2	(10 μM)	19.5 ± 2.9	21.5 ± 4.6	36.7 ± 2.5
20:4 + H_2O_2	(50 μM)	20.8 ± 4.3	22.7 ± 3.0	40.0 ± 3.6
20:4 + H_2O_2	(10 μM)	22.0 ± 3.9	25.3 ± 4.6	37.5 ± 4.5
20:4 + H_2O_2	(200 μM)	25.8 ± 2.2	25.1 ± 2.7	33.1 ± 3.8

DISCUSSION

Guanylate cyclase catalyses the formation of cGMP and occurs in two different forms. One is membrane bound and coupled to a receptor, the second is soluble and has a heme regulatory subunit. Our previous results on the stimulation of sGC by CO[27] were corroborated in the present study with a partially purified enzyme. Since the effect was light-reversible and the preparation showed a spectral shift after addition of CO we interprete the stimulation by the binding of CO to the heme-regulatory subunit of sGC. The wellknown stimulator NO (+ EDRF) also interacts strongly with ferrous hemoproteins so that this ligand could exert the stimulation in an analogous way. It should be mentioned, however, that the Soret band in our preparation was located at 415 nm and not at 430 nm as reported for sGC of bovine lung.[32] A contamination with hemoglobin can be ruled out since sodium dithionite addition did not change the spectrum. This would have been the case in the presence

of either oxy- or methemoglobin. An activation of a hemoprotein by CO must be regarded as unusual since so far all effects of CO on hemoproteins are based on a competition with dioxygen binding. For an involvement of dioxygen no indications were found. One possible mechanism of action was that in our system CO prevented an inactivation by another yet unknown agent. Superoxide anions were described as modulators of GC-activity. Indeed, platelets made insensitive by CO towards aggregating agonists like arachidonate, collagen or thrombin could be activated in the presence of an $O_2^{\cdot -}$-generating system (B. Brüne, unpublished). Its action could be traced back to reduced cGMP-levels when a platelet homogenate was stimulated with CO. However, no significant stimulation of GC-activity could be detected using SOD alone. A first possible explanation of this surprising result might be that a high affinity of GC for $O_2^{\cdot -}$ as used in our experiments was lowered in the presence of CO. This would allow SOD only to act on the CO-protected enzyme. On the other hand, the inhibition of the enriched enzyme by enzymatically generated O_2-was fully responsive to SOD so the lack of a protective effect of SOD in the 100,000 × g supernatant remains unclear.

Our findings could have profound implications for the pathogenesis of reperfusion injury. Bednar *et al.*[34] reported that ^{111}In-labelled platelet accumulations in infarcted myocardium are due to PMN activation. If the $O_2^{\cdot -}$ generation is triggered by a still unknown mechanism, a chain of fatal events arises: in platelets the cGMP levels decrease and, depending on the concentrations of $O_2^{\cdot -}$, increase the sensitivity for aggregation or directly lead to first adherence and then aggregation. At the same time, the endothelium may contract in response to thrombin and histamine[35,36] and expose collagen fibres which potently activate platelets. Platelets and PMN can further synergistically stimulate each other by the formation of platelet activating factor (PAF) and a host of secretory products. The formation of TxA_2 as a vasoconstricting mediator and stimulator of PMN adherence contributes to the multifactorial event of vessel occlusion necrosis. Superoxide, however, should have a second mechanism of action by effectively scavenging the radical NO released from endothelial cells.

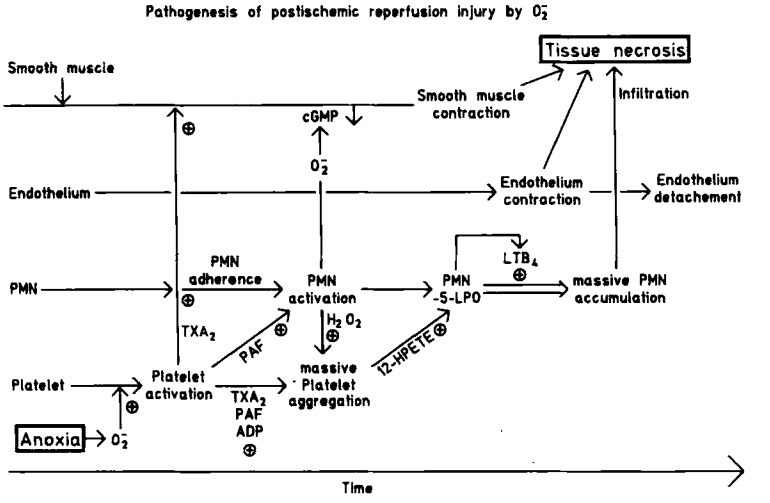

FIGURE 3 Proposed mechanisms during reperfusion injury involving $O_2^{\cdot -}$.

Therefore, not only the direct inhibition of sGC is a consequence but also the stimulatory influence of NO would be eliminated. A schematic presentation of these possible mechanisms is summarized in the above scheme.

The series of events is by far not complete, since not only platelet G-cyclase can be affected but that of other cells as well. Since the functions of cGMP are poorly investigated, one cannot deduce possible effects in other cells. Generally speaking however, cGMP causes a desensitization of cells towards activation.

The second reactive oxygen species that is supposed to interfere with platelet aggregation is H_2O_2. Mounting data exist that this peroxide can play a particular role during platelet activation. Proaggregatory as well as inhibiting properties are discussed.[23–25] We concentrated on the stimulating effects of H_2O_2 during cell activation. Simultaneous addition of subthreshold concentrations of agonists and hydrogen peroxide caused a complete platelet aggregation, secretion and onset of arachidonic acid metabolism. These stimulating properties were completely abolished by the addition of the cyclooxygenase inhibitors, aspirin and indomethacin or the thromboxane/endoperoxide receptor antagonist BM 13.177. These data implicate that the stimulating properties of H_2O_2 involve cycloxygenase activation. Hemler and Lands[37] described the peroxide-dependent activation of cyclooxygenase. These results were confirmed by Vargaftig *et al.*[38] Thus, one major property of H_2O_2 seems to be facilitating the formation of PGH_2 by cyclooxygenase and thus providing the substrate for thromboxane synthase. Its product TxA_2 is considered to be the most potent physiological proaggregatory eicosanoid. To our knowledge, the results introduced here demonstrate for the first time that one of the major roles of H_2O_2 lies in the potentiation of effects elicited by subthreshold stimulation of platelets. Thus, the neutrophil-derived H_2O_2 might shift the complexly regulated steady state of thrombosis and hemostasis towards clot formation and small vessel occlusion.

Together with the new postulated role of O_2^- by inhibition of guanylate cyclase, we suggest that the influence of the oxidative burst of PMN in the phenomenon of reperfusion injury should be reconsidered.

Acknowledgement

The skillful technical assistance of Miss B. Diewald is gratefully acknowledged. We also thank the DRK-Ulm for a continuous supply of platelet-rich plasma.

References

1. Simpson, P.J., Mickelson, J.K. and Lucchesi, B.R. *Fed. Proc.*, **46**, 2413–2421, (1987).
2. Boveris, A. and Chance, B. *Biochem. J.*, **134**, 707–716, (1973).
3. Otani, H., Tanaka, H., Inoue, T., Umemoto, M. and Omoto, K. *Circ. Res.*, **55**, 168–175, (1984).
4. Granger, D.N., Rutili, G. and McCord, J.M. *Gastroenterol.*, **78**, 474–480, (1981).
5. Chambers, D.E., Parks, D.A., Patterson, G., Roy, R. and McCord, J.M. *J. Mol. Cell. Cardiol.*, **17**, 145–152, (1985).
6. Hearse, D.J., Manning, A.S., Downey, J.M. and Yellon, D.M. *Acta Physiol. Scand.*, **548**, 65–76, (1986).
7. Lucchesi, B.R. and Mullane, K.M. *Ann. Rev. Pharmacol. Toxicol.*, **26**, 201–247, (1986).
8. Mruxfeldt, M. and Sharper, W. *Basic Res. Cardiol.*, **82**, 486–492, (1987).
9. Werns, S.W., Shea, M.J., Mitsos, S.E., Dysko, R.C., Fantone, J.C, Schork, M.A., Anrams, G.D., Pitt, B. and Lucchesi, B. *Circ.*, **73**, 518–524, (1986).
10. Manfedi, J.P. and Holmes, E.W. *Annu. Rev. Physiol.*, **47**, 691–705, (1985).

11. Bevilacqua, M.P., Pober, J.S., Mendrick, D.L., Cotran, M.S. and Gimbrone, M.A. Jr.: *Proc. Natl. Acad. Sci. USA*, **84**, 9238–9242, (1987).
12. Hallett, M.B., Shandall, A. and Young, H.L. *Biochem. Pharmacol.*, **34**, 1757–1761, (1985).
13. Mullane, K.M., Salmon, J.A. and Kraemer, R. *Fed. Proc.*, **46**, 2422–2433, (1987).
14. Mercandetti, R.J., Lane, T.A. and Colmerauer, M.E.M. *J. Lab. Clin. Med.*, **104**, 370–380, (1984).
15. Farber, H.W., Center, D.M. and Rounds, S. *Circ. Res.*, **57**, 898–902, (1985).
16. Maroko, P.R., Carpenter, C.B., Chiarioello, M., Fishbein, M.C. Radvany, P., Knostman, J.D. and Haie, S.L. *J. Clin. Invest.*, **61**, 661–670, (1978).
17. Engler, R.L., Schmid-Schönbein, R.W. and Pavelec, R.W. *Am. J. Pathol.*, **111**, 98–111, (1983).
18. Romson, J.L., Hock, B.G., Kunkel, S.L., Abrams, G.D., Schork, M.A. and Lucchesi, B.R.: *Circ.*, **67**, 1016–1023, (1983).
19. Mullane, K.M., Read, N., Salmon, J.A. and Moncada, S. *J. Pharmacol: Exp. Ther.*, **228**, 510–522, (1984).
20. Bolli, R., Cannon, R.O., Speri, E., Goldstein, R.E. and Epstein, S.E.*J. Amn. Coll. Cardiol.*, **2**, 671–688, (1983).
21. Meerson, F.Z., Kagan, V., Kozlov, P., Belkin, L.M. and Arkhipenko, V. *Basic Res. CArdiol.*, **77**, 465–485, (1982).
22. Stam, H. and Koster, J. In: Prostaglandins and other eicosanoids in the cardiovascular system. Schrör, K. (ed.) Karger-Basel: 131–148, (1985).
23. Rodvien, R., Lindon, J.N. and Levine, P.H. *Br. J. Haematol.*, **33**, 19–26, (1976).
24. Canoso, R.T., Rodvien, R., Scoon, K. and Levine, P.H. *Blood*, **43**, 645–656.
25. Del Principe, D., Menichelli, A., De Matteis, W., Di Corpo, M.L., Di Guilio, S. and Finazzo – Agro, A. *FEBS Lett.*, **185**, 142–146, (1985).
26. Handin, R.J., Karabin, R. and Boxer, G.J. *J. Clin. Invest.*, **59**, 959–965, (1977).
27. Palmer, R.M.J., Berrige, A.G. and Moncada, S. *Nature* (*London*), **327**, 524–526, (1987).
29. Mülsch, A., Naunym-Schneiedeberg's *Arch. Pharmacol.* **338**, 147 (1988).
30. Siess, W., Weber, P.C. and Lapetina, E.G. *J. Biol. Chem.*, **259**, 8286–8292, (1984).
31. Born, G.V.R. *Nature*, **194**, 927–929, (1962).
32. Gerzer, R., Böhme, E., Hofmann, F. and Schultz, G. *FEBS-Lett.*, **132**, 71–74, (1981).
33. Freeman, B.A. and Crapo, J.D. *Lab. Invest.*, **47**, 412–425, (1982).
34. Bednar, M., Smith, B., Pinto, A. and Mullane, K.M. *J. Cardiovasc. Pharmacol.*, **7**, 906–912, (1985).
35. De Clerck, F., De Brabander, M., Neels, H. and Van de Velden, V. *Thromb. Res.*, **23**, 505–520, (1981).
36. Hammersen, F., Hammersen, E. and Osterkamp-Baust, U. In: Bau and Funktion endothelialer Zellen. K. Messmer, F. Hammersen (Eds), Karger–Basel, 1–18, (1983).
37. Hemler, M.E. and Lands, W.E.M. *J. Biol. Chem.*, **255**, 6253–6261, (1980).
38. Vargaftig, B.B., Tranier, Y. and Chignard, M. *Eur. J. Pharmacol.*, **33**, 19–29, (1978).

Accepted by Prof. T.F. Slater