ELSEVIER

Cardiovascular Research 73 (2007) 568 – 574

Cardiovascular Research

www.elsevier.com/locate/cardiores

The role of poly(ADP-ribose) polymerase (PARP) in the autonomous proliferative response of endothelial cells to hypoxia☆

Yaser Abdallah *, Dragan Gligorievski, Sascha A. Kasseckert, Lukas Dieterich, Matthias Schäfer, Christoph R. Kuhlmann, Thomas Noll, Heinrich Sauer, H.Michael Piper, Claudia Schäfer

Institute of Physiology, Justus-Liebig-University, D-35392 Giessen, Germany

Received 31 July 2006; received in revised form 3 November 2006; accepted 20 November 2006
Available online 23 November 2006
Time for primary review 34 days

Abstract

Objective: The autonomous proliferative response of endothelial cells to hypoxia has been shown to be dependent on activation of NAD(P)H oxidase, on the cytosolic Ca^{2+} load, and, consequently, on nuclear translocation of extracellular signal-regulated kinase (ERK)1/2 during transient hypoxia. The aim of the present study was to investigate whether poly(ADP-ribose) polymerase (PARP) is a downstream signal of NAD(P)H oxidase, mediating cytosolic Ca^{2+} load and hence nuclear translocation of ERK1/2 and endothelial cell proliferation.
Methods: Porcine aortic endothelial cells were incubated under hypoxic conditions for 40 min. Cytosolic [Ca^{2+}] and reactive oxygen species (ROS) formation were measured in fura-2- and DCF-loaded cells, respectively. PARP activation was detected by immunocytochemistry, and endothelial cell proliferation was determined 24 h after 60 min of transient hypoxia.
Results: Inhibition of NAD(P)H oxidase with antisense oligonucleotide against the $p22^{phox}$ subunit, MEK/ERK signalling with UO 126 (30 μM), or PARP with PJ 34 (10 μM) leads to a marked reduction in hypoxia-induced cytosolic Ca^{2+} load and activation of PARP. Hypoxia-induced translocation of ERK1/2 and endothelial cell proliferation were also prevented when NAD(P)H oxidase or PARP were inhibited; however, hypoxic ROS formation was not affected in the presence of PARP inhibitor.
Conclusion: PARP represents a downstream effector of NADP(H) oxidase and acts as a necessary intermediate step for the hypoxic proliferative response of endothelial cells.

Keywords: PARP; Hypoxia; Endothelial cell; Proloferation; Calcium; ROS

1. Introduction

Proliferation of endothelial cells is a key step in angiogenesis. Earlier studies of our group showed that short term hypoxia induces an autonomous proliferative response of endothelial cells. This autonomous response is not due to the paracrine or autocrine actions of mediators of tissue hypoxia such as vascular endothelial growth factor or adenosine, but to the interplay of two endogenous signalling mechanisms in hypoxic endothelial cells, one related to generation of reactive oxygen species (ROS) and the other to a rise of cytosolic [Ca^{2+}] [1,2].

With respect to the first proliferation-stimulating mechanism, it was demonstrated that small amounts of ROS derived from mitochondria during hypoxia cause an activation of the MEK/ERK pathway, which in turn activates the NAD(P)H oxidase complex and thereby amplifies markedly the generation of ROS in endothelial cells. Antisense oligonucleotides directed against $p22^{phox}$, an essential subunit of the NAD(P)H oxidase complex, suppressed the larger part of hypoxic generation of ROS and inhibited hypoxia-induced cell proliferation [1].

With respect to the second mechanism, it was shown that the autonomous proliferative response of endothelial cells to

☆ Hiroshi Watanabe (Hamamatsu University, Japan).

* Corresponding author. Physiologisches Institut Justus-Liebig-Universität Aulweg 129 D-35392 Giessen Germany. Tel.: +49 6419947223; fax: +49 6419947219.
E-mail address: Yaser.Abdallah@physiologie.med.uni-giessen.de (Y. Abdallah).

0008-6363/$ - see front matter © 2006 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.cardiores.2006.11.027

hypoxia depends on the hypoxic rise of cytosolic Ca^{2+} [2]. Once endothelial cells are exposed to hypoxia, cytosolic Ca^{2+} rises in two phases: Initially, cytosolic Ca^{2+} rises transiently due to a release from the endoplasmic reticulum. This phase is followed by a sustained Ca^{2+} rise due to an influx of Ca^{2+} from the exterior space across the plasmalemma [3,4]. This sustained Ca^{2+} rise was found to be essential for a nuclear translocation of extracellular signal-regulated kinase (ERK)1/2, which in turn is required for the proliferative response to hypoxia [2].

The central hypothesis of the present study was that NAD(P)H oxidase activation triggers the sustained hypoxic Ca^{2+} overload and subsequent nuclear translocation of ERK1/2 in endothelial cells.

In this context, the role of poly(ADP-ribose) polymerase (PARP) in these signalling mechanisms was investigated. The interest in the role of PARP arose because, first, PARP is activated in response to oxidative stress in a variety of cell types and, second, free ADP-ribose, i.e. the product of PARP activation is able to gate Ca^{2+}-permeable cation channels in cells expressing specific transient receptor potential (TRP) proteins [5,6]. It has also been shown in a TRP-expressing cell line that ROS-induced Ca^{2+} influx can be prevented in the presence of PARP inhibitors [7]. Third, PARP has been found to be involved in various proliferative mechanisms of vascular cells [8,9].

2. Materials and methods

2.1. Endothelial cell isolation and culture

Endothelial cells were isolated from porcine aorta as described before [10]. The porcine aortas were obtained from a local slaughterhouse. Freshly isolated cells were grown in culture using M199 supplemented with 10% fetal calf serum and 1% penicillin/streptomycin. Porcine aorta endothelial cells (PAEC) were trypsinized after they had reached confluence, and plated on glass cover slips, or plastic culture dishes.

2.2. Media

The HEPES-buffered medium contained (mM): 125.0 NaCl, 2.6 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.0 $CaCl_2$, and 25.0 HEPES. For normoxia, medium pH_o was adjusted to 7.4 and 2.5 mM glucose was added. For hypoxia, medium was autoclaved and gassed with 100% N_2, glucose was omitted, and pH_o was adjusted to 6.4.

2.3. Experimental protocols

After 10 min normoxic incubation (pH 7.4) cells were incubated for 60 min under hypoxic conditions (N_2/pH 6.4). The specific PARP inhibitor, PJ 34 (10 μM) or MEK/ERK inhibitor, UO 126 (30 μM) were added 5 min before and during hypoxia. Nonsense (NS) or antisense (AS) oligonucleotides against $p22^{phox}$ subunit of NAD(P)H oxidase were added 24 h before beginning the experiment.

2.4. Ca^{2+} and ROS measurement

Cytosolic concentration of Ca^{2+} was measured with the fluorescent indicator fura-2. Cells were incubated with the acetoxymethyl ester of fura-2 (2.5 μM) for 60 min in medium M199. This was followed by 30 min incubation without fura-2. Fura-2 fluorescence was analyzed using a TILL Photonics imaging system (Martinsried, Germany). Excitation was alternated between 340 and 380 nm. Emitted light was detected at 510 nm, and the background was corrected. Fura-2 fluorescence was calibrated according to the methods described by Grynkiewicz et al. [11].

For ROS measurements, cells were loaded with 2′,7′-dichlorofluorescein diacetate (DCF; 10 μM). The DCF signal was detected using the 475 nm excitation wavelength. The formation of ROS was expressed as arbitrary units of DCF fluorescence signal.

2.5. Cell proliferation

For cell proliferation, subconfluent monolayers were washed and incubated for 24 h in serum-free media to arrest cell proliferation. After 60 min hypoxia cells were incubated for a further 24 h. To determine cell proliferation, cells were then trypsinized and counted in a Neubauer chamber.

2.6. Immunocytochemical analysis of p42/p44 MAPK-activation

After 1 h hypoxic incubation cells were treated with ice-cold 100% methanol. After fixation, cells were permeabilized with PBS containing 0.1% Tween and were subsequently blocked with PBS/BSA blocking solution for 1 h at room temperature. After blocking, cells were incubated with the primary antibody directed against the phosphorylated ERK1/2 (1:200) for 1 h at room temperature, followed by a washing step with PBS. Cells were then incubated with a biotylinated secondary antibody (1:50000) for 30 min followed by extensive washing steps with PBS. Finally, cells were incubated with Texas Red conjugated Streptavidin (1:800) overnight after which they were washed with PBS, mounted on microscope slides and fluorescence was detected with a fluorescence microscope.

2.7. Immunocytochemical analysis of PARP activation

For the analysis of PARP activation, we used the immunocytochemical analysis of PAR, the enzymatic end-product of PARP activation. Experimental incubations were stopped with 10% TCA (trichloroacetic acid) at room temperature and then permeabilized with PBS containing 0.1% Tween 20. Cells were then blocked with a PBS/BSA blocking solution for 1 h at room temperature after which they were incubated with the primary antibody (1:300) for 1 h at room temperature. Primary antibody incubation was followed by extensive washes with PBS. Cells were

subsequently incubated with the biotin conjugated anti-mouse IgG secondary antibody (1:10000) for 30 min at room temperature. Following washes with PBS, Texas Red Conjugated Streptavidin (1:800) (Rockland) was used as chromogen overnight at room temperature after which cells were extensively washed with PBS and mounted on microscope slides. Fluorescence was detected under fluorescence microscopy using the Till Vision Software. The Excitation wavelength for Texas Red was 595 nm.

2.8. Antisense transfection experiments

We used an antisense oligonucleotides approach to down regulate the expression of $p22^{phox}$, an essential subunit of the NAD(P)H oxidase. Endothelial monolayers were incubated for 24 h (500 nM) with two different overlapping sequences of 16-mer length, which were phosphorothioated. Sequence of $p22^{phox}$ antisense oligonucleotides : 5′-TCTGTCCCATGGC-GAT-3′; of nonsense oligonucleotides: 5′-TGTCCCATGGC-GATGC-3′.

3. Statistics

Data are given as mean values ± SEM from individual cells investigated in separate experiments. Statistical comparisons were performed by one-way ANOVA and use of the Student–Newman–Keuls test for post hoc analysis. Differences with $p < 0.05$ were considered as statistically significant.

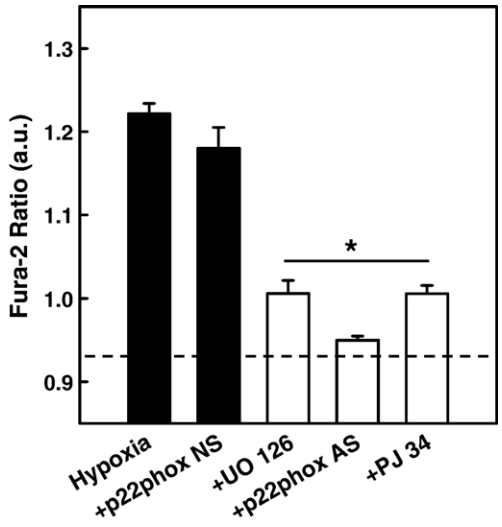

Fig. 2. Statistical analysis of cytosolic $[Ca^{2+}]$ after 60 min of hypoxia under control conditions (hypoxia), in $p22^{phox}$ nonsense treated cells, in the presence of MEK/ERK inhibitor (UO 126), in $p22^{phox}$ antisense treated cells, or in the presence of the PARP inhibitor (PJ 34). Dashed line represents normoxic values. Fura-2 ratio is expressed in arbitrary units. Data are mean ± SEM. $n = 6$ experiments. $^*p < 0.05$ vs. hypoxia.

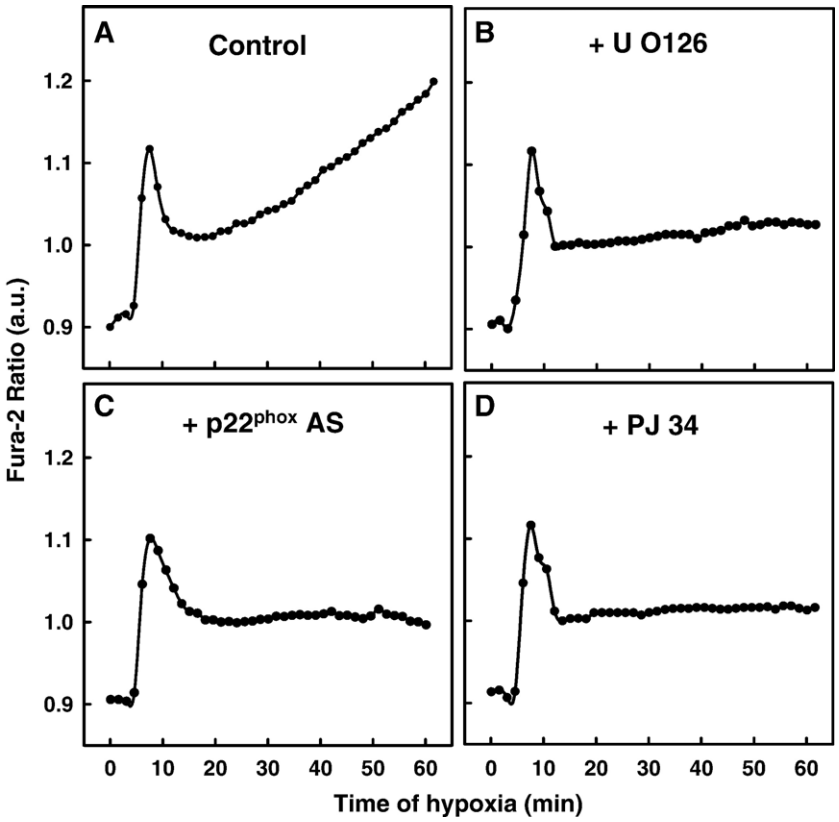

Fig. 1. A–D: Representative traces of cytosolic $[Ca^{2+}]$ during 60 min of hypoxia in under control conditions (hypoxia), in the presence of MEK/ERK inhibitor (UO126), in $p22^{phox}$ antisense treated cells, or in the presence of the PARP inhibitor (PJ 34).

4. Results

4.1. The role of MEK/ERK signalling, NAD(P)H oxidase, and PARP activation for hypoxic Ca^{2+} load

When endothelial cells were exposed to 60 min of transient hypoxia, cytosolic Ca^{2+} rises in two phases (Fig. 1A): First, a transient increase in cytosolic Ca^{2+} within the first 15 min, which is due to a release from the endoplasmic reticulum. Second, a sustained cytosolic Ca^{2+} rise due to an influx of Ca^{2+} from the extracellular space [3,4]. We showed in previous studies that the second sustained Ca^{2+} rise is essential for a nuclear translocation of ERK1/2, which is required for the proliferative response of endothelial cells to hypoxia [2]. In the present study, we investigated whether the increase in cytosolic Ca^{2+} depends on activation of MEK/ERK signalling, NAD(P)H oxidase, or PARP. Firstly, we tested whether sustained cytosolic Ca^{2+} overload is dependent on activation of ERK1/2. UO 126, an inhibitor of the MEK/ERK signalling was applied during the hypoxic period. When MEK/ERK signalling was inhibited, the second sustained hypoxic Ca^{2+} overload was markedly reduced, while the transient rise remained unaffected (Fig. 1B).

Secondly, cells were pre-treated with antisense oligonucleotides (AS) against the $p22^{phox}$ subunit of NAD(P)H oxidase. As shown in (Fig. 1C), inhibition of NAD(P)H oxidase did not affect the initial transient rise of cytosolic

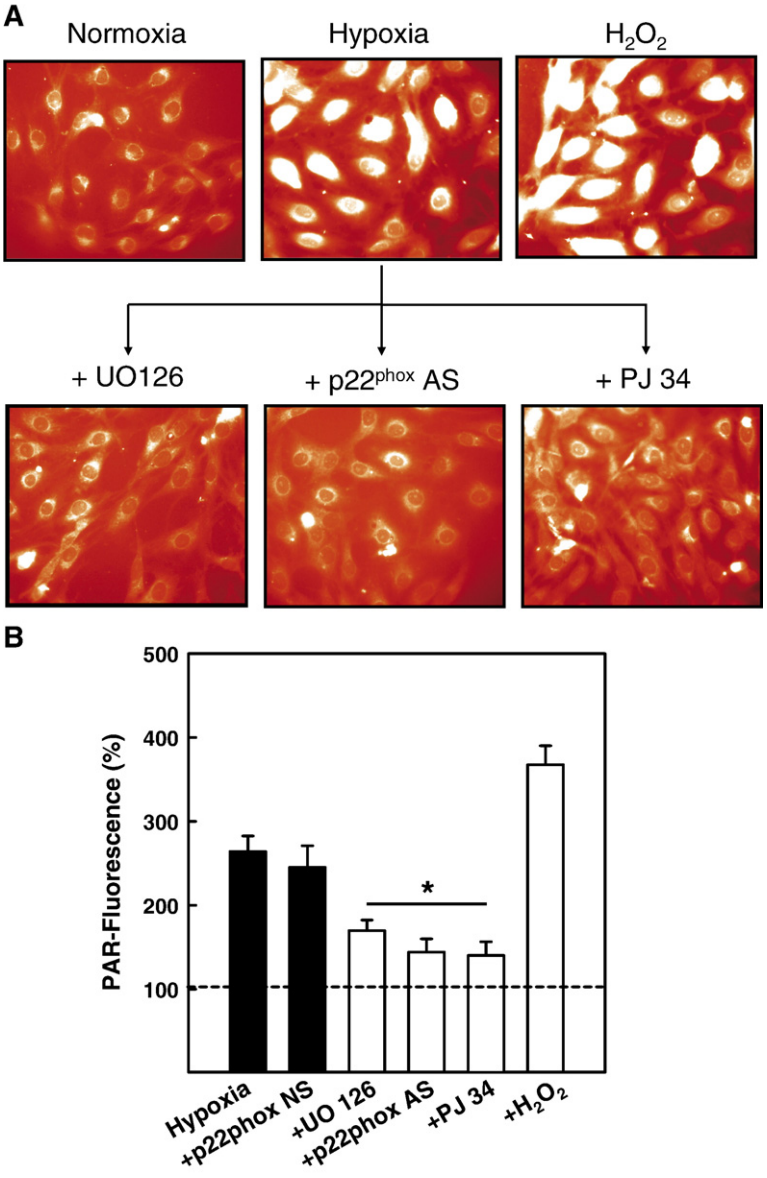

Fig. 3. Upper panel (A): Representative micrographs indicating PAR fluorescence under normoxic conditions, 60 min hypoxia, hypoxia+MEK/ERK inhibitor (UO 126), hypoxia+$p22^{phox}$ antisense treatment, hypoxia+PARP inhibitor (PJ 34), or application of 30 μM H_2O_2. Lower panel (B): Statistical analyses of PAR fluorescence. Normoxic values were set to 100% (dashed line). Data are mean±SEM. $n=6$ experiments. *$p<0.05$ vs. hypoxia.

Ca^{2+}, while the secondary sustained rise of Ca^{2+} was markedly reduced. Pre-treatment with $p22^{phox}$ nonsense oligonucleotides (NS) did not affect the cytosolic Ca^{2+} kinetics during hypoxia. These findings demonstrate therefore that the transplasmalemmal Ca^{2+} influx depends on activation of NAD(P)H oxidase.

Thirdly, the role of PARP as a potential downstream signal of NAD(P)H for the sustained cytosolic Ca^{2+} overload was tested. As shown in Fig. 1D; the presence of the PARP inhibitor, PJ 34 during hypoxia reduced significantly the sustained Ca^{2+} overload without affecting the initial transient Ca^{2+} rise.

The statistical analysis of Ca^{2+} data at 60 min hypoxia under the different treatments are summarized in Fig. 2. Vehicle experiments with DMSO at a final concentration of 0.01% (v/v) did neither affect the transient nor the sustained Ca^{2+} rise in hypoxic endothelial cells (fura-2 ratio after 60 min hypoxia; control: 1.22±0.02; vehicle: 1.18±0.03; $n=4$ experiments).

4.2. Hypoxia-induced activation of PARP

At the second point of our analysis we investigated whether hypoxic PARP activation could be affected by MEK/ERK signalling or NAD(P)H oxidase. PARP activation was monitored by measuring poly(ADP) ribose (PAR) using immunocytochemistry. As indicated in Fig. 3A, 60 min of hypoxic incubation caused an increase in PAR fluorescence to 2.6-fold of the initial value. For comparison, the application of H_2O_2 (90 μM) caused a 3.6-fold rise in PAR fluorescence. Presence of the specific PARP inhibitor, PJ 34 (10 μM) prevented the hypoxic activation of PARP. PARP activation during hypoxia was also prevented when cells had been pre-treated with antisense oligonucleotides against $p22^{phox}$ or when the MEK/ERK inhibitor UO 126 had been present. Pre-treatment with $p22^{phox}$ nonsense oligonucleotides (NS) did not affect the hypoxia-induced PARP activation. The statistical analysis of PARP activation at 60 min hypoxia under the different treatment are summarized in Fig. 3B. These results show that hypoxia-induced PARP activation indeed depends on activation of MEK/ERK and NAD(P)H oxidase.

4.3. ROS generation during hypoxia

As the third point, we were interested in the downstream positioning of PARP relative to NAD(P)H oxidase. The latter was confirmed by measurement of hypoxic ROS generation using DCF fluorescence in the presence of the PARP inhibitor, PJ 34 or AS pre-treatment of the NAD(P)H oxidase. As shown in Fig. 4, hypoxia leads to an increase in ROS indicated by a rise of the DCF signal. Hypoxia-induced ROS generation was significantly reduced in $p22^{phox}$ antisense pre-treated cells, but not in the presence of the PARP inhibitor PJ 34 or when cells were pre-treated with

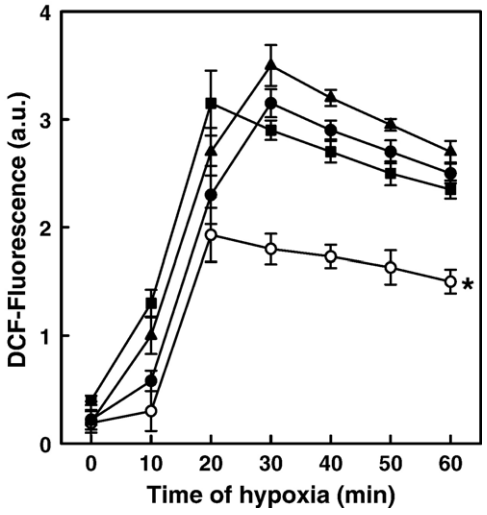

Fig. 4. Time course of ROS generation (DCF fluorescence) during 60 min hypoxia under control conditions (▲), in $p22^{phox}$ nonsense-treated cells (■), in $p22^{phox}$ antisense treated cells (○), and in the presence of PARP inhibitor, PJ 34 (●). Data are mean±SEM. $n=6$ experiments. *$p<0.05$ vs. all other conditions.

$p22^{phox}$ nonsense oligonucleotides. This indicates that PARP activation is localized as a down-stream of NAD(P)H oxidase.

4.4. Nuclear translocation of ERK1/2

The fourth point of our analysis concerned the role of NAD(P)H oxidase and PARP for nuclear translocation of ERK1/2, which was analyzed by the use of immunocytochemistry. As shown in the representative micrographs in Fig. 5A, ERK1/2 was located predominantly in the nuclei after 60 min of hypoxia. When cells were either pre-treated with $p22^{phox}$ antisense oligonucleotides or a PARP inhibitor, PJ 34, was applied during hypoxia, ERK1/2 remains in a perinuclear localisation, i.e. the nuclear translocation of ERK1/2 was prevented (Fig. 5A). The statistical analysis of nuclear ERK1/2 translocation, i.e. nuclear/cytosolic fraction is summarized in Fig. 5B. Pre-treatment with $p22^{phox}$ nonsense oligonucleotides did not affect the nuclear translocation of ERK1/2 during hypoxia. This finding indicates that the translocation of ERK1/2 is located as a downstream of NAD(P)H oxidase and PARP.

4.5. Cell proliferation

As the fifth and final step of our study we tested whether the investigated signalling pathway consisting of NAD(P)H oxidase and PARP is involved in hypoxia-induced endothelial cell proliferation. Cells were subjected to a transient period of hypoxia (60 min) and then incubated for 24 h under normoxic condition. As shown in Fig. 6, 60 min of transient hypoxia leads to an increase of endothelial cell proliferation.

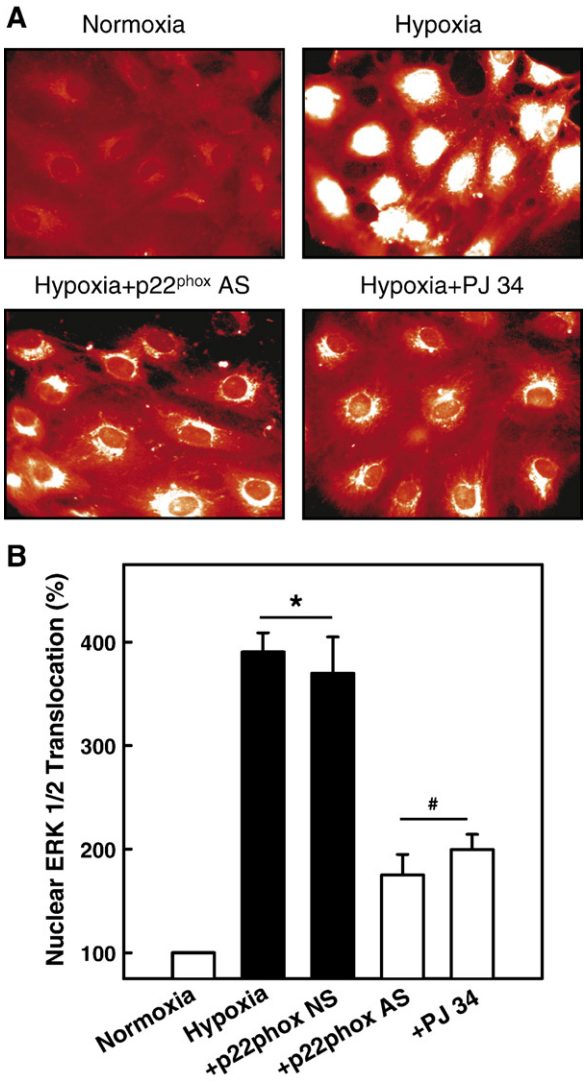

Fig. 5. Upper panel (A): Representative micrographs showing localisation of ERK1/2 under normoxic condition, 60 min of hypoxia, hypoxia+p22^{phox} antisense treatment, or hypoxia+PARP inhibitor (PJ 34). Lower panel (B): Statistical analyses of nuclear ERK1/2 translocation. Normoxic values were set to 100%. Data are mean±SEM. $n=6$ experiments. *$p<0.05$ vs. normoxia, $^{\#}p<0.05$ vs. hypoxia and hypoxia+p22^{phox} nonsense treatment.

Both p22^{phox} antisense oligonucleotides treatment or PARP inhibition abolished the proliferative response of endothelial cells to transient hypoxia, while the latter was not significantly affected, when cells were treated with p22^{phox} nonsense oligonucleotides.

5. Discussion

The aim of the present study was to analyse the signalling of the autonomous proliferative response of endothelial cells to hypoxia. In this context we focused on one hand on the relationship between activation of NAD(P)H oxidase and sustained cytosolic Ca^{2+} overload during hypoxia as an essential trigger for nuclear translocation of ERK1/2, and on the other on the role of PARP within this scenario.

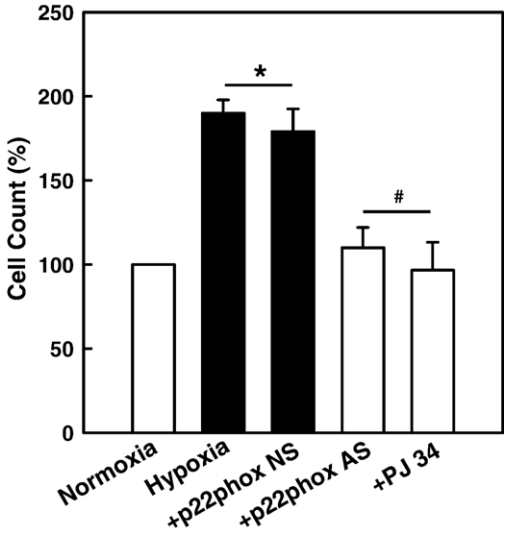

Fig. 6. Endothelial cell proliferation (cell count) after 24 h under normoxic conditions, after 60 min of transient hypoxia, hypoxia+p22^{phox} nonsense treatment, hypoxia+p22^{phox} antisense treatment, or hypoxia+PARP inhibitor (PJ 34). Normoxic values were set to 100%. $n=6$ experiments. *$p<0.05$ vs. normoxia, $^{\#}p<0.05$ vs. hypoxia and hypoxia+p22^{phox} nonsense.

The principal findings are that activation of ERK1/2 and NAD(P)H oxidase trigger the sustained cytosolic Ca^{2+} rise and, secondarily, the nuclear translocation of ERK1/2, a signal previously shown to initiate the proliferative response of endothelial cells [1,2]. The data also show that activation of PARP is located in a downstream position relative to NAD(P)H oxidase and upstream to cytosolic Ca^{2+} overload, and that it represents a necessary intermediate step for the hypoxic proliferative response.

In hypoxic endothelial cells, cytosolic Ca^{2+} overload develops in two phases: Within the first minutes of hypoxia, cytosolic Ca^{2+} rises transiently. As we showed previously, this is due to an IP_3-dependent Ca^{2+} release from the endoplasmic reticulum [3,4]. This phase is followed by a second one characterized by a progressive and sustained rise of cytosolic Ca^{2+}. This second phase is dependent on the initial emptying

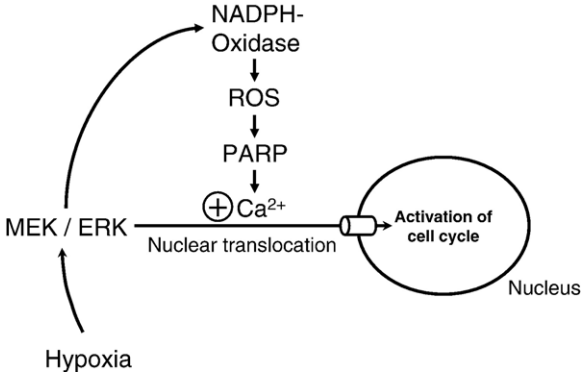

Fig. 7. Signalling scheme of autonomous proliferative response to hypoxia in endothelial cells.

of the endoplasmic reticulum and caused by an influx of Ca^{2+}across the plasmalemma [3]. It is likely due to the opening of store-operated cation channels in the plasmalemma.

In the present study, we now demonstrate that the second Ca^{2+} rise, i.e. the Ca^{2+} influx, is triggered by a prior activation of NAD(P)H oxidase and PARP. Our data do not identify the exact mechanism by which PARP can induce the cytosolic Ca^{2+} overload, but it seems likely that the product of PARP activation, PAR, targets TRP proteins, which constitute store-operated channels on the molecular level. Whole-cell and single-channel analysis of cells expressing specific TRP protein, showed that these can function as Ca^{2+}-permeable cation channels which are specifically gated by ADP-ribose, i.e. a product of PARP activation [5,6]. It has also been shown in a TRP expressing cell line that ROS-induced Ca^{2+} influx can be prevented by the presence of PARP inhibitors [7]. At least for the endothelial cells here investigated the ROS-induced Ca^{2+} influx cannot be attributed to an oxidative modification of TRP proteins, as this would not require the participation of PARP, which here was shown to be a prerequisite for the ROS-induced Ca^{2+} influx.

During hypoxia, PARP was activated with a maximum peak after 60 min. The hypoxia-induced PARP activation was dependent on a prior activation of NAD(P)H oxidase, which could mean that PARP may become activated through ROS-induced DNA strand breaks; however, the latter was not investigated in the present study. Alternatively, PARP could become activated by an IP_3-dependent increase in nuclear Ca^{2+}, independent of occurrence of DNA strand breaks, as found by other investigators [12]. In our model, we showed previously that the first transient increase in cytosolic Ca^{2+} during hypoxia is sensitive to inhibitors of the IP_3 release channel on the endoplasmic reticulum of endothelial cell [3,4]. One could hypothesize that the first transient Ca^{2+} release causes an increase in nuclear Ca^{2+} and thus PARP activation, but this was not in the scope of the present study.

Beside its main biological role, i.e. repair of DNA strand breaks and maintenance of genomic integrity, PARP was found to be involved in replication and cell differentiation. This observation is supported by the findings that i) PARP is highly activated in the nuclei of proliferating cells, ii) PARP is part of the multiprotein replication complex (MRC), and iii) PARP facilitates the assembly and deposition of histone complexes on DNA during replication [13,14]. In contrast to these nuclear roles of PARP, we show here that hypoxia-induced PARP activation is an essential step for cell proliferation induced by transient hypoxia through increasing cytosolic Ca^{2+} concentration and the subsequent translocation of ERK1/2 into the nucleus. In other words, PARP activation can modulate the cell cycle by targeting extra-nuclear effectors, i.e. cytosolic Ca^{2+}, which in turn induces the translocation of ERK 1/2 into the nucleus.

Our principal findings are summarized in Fig. 7: The hypoxia-induced activation of ERK1/2 leads to an increase in NADP(H) oxidase activity and hence ROS generation. In the following step, it is concluded that ROS induce the sustained cytosolic Ca^{2+} raise through activation of PARP. The sustained Ca^{2+} rise then triggers the translocation of ERK1/2 into the nucleus, initiating cell cycle activation.

Since hypoxia-induced endothelial cell proliferation plays an important role for angiogenesis in solid tumours and, hence, their growth, a PARP antagonist may represent a new agent for cancer therapies targeting the tumour vasculature.

References

[1] Schafer M, Schafer C, Ewald N, Piper HM, Noll T. Role of redox signaling in the autonomous proliferative response of endothelial cells to hypoxia. Circ Res 2003;92:1010–5.
[2] Schafer M, Ewald N, Schafer C, Stapler A, Piper HM, Noll T. Signaling of hypoxia-induced autonomous proliferation of endothelial cells. FASEB J 2003;17:449–51.
[3] Ladilov Y, Schafer C, Held A, Schafer M, Noll T, Piper HM. Mechanism of Ca(2+) overload in endothelial cells exposed to simulated ischemia. Cardiovasc Res 2000;47:394–403.
[4] Schafer M, Bahde D, Bosche B, Ladilov Y, Schafer C, Piper HM, et al. Modulation of early [Ca2+]i rise in metabolically inhibited endothelial cells by xestospongin C. Am J Physiol Heart Circ Physiol 2001;280: H1002–10.
[5] Perraud AL, Fleig A, Dunn CA, Bagley LA, Launay P, Schmitz C, et al. ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. Nature 2001;411:595–9.
[6] Inamura K, Sano Y, Mochizuki S, Yokoi H, Miyake A, Nozawa K, et al. Response to ADP-ribose by activation of TRPM2 in the CRI-G1 insulinoma cell line. J Membr Biol 2003;191:201–7.
[7] Fonfria E, Marshall IC, Benham CD, Boyfield I, Brown JD, Hill K, et al. TRPM2 channel opening in response to oxidative stress is dependent on activation of poly(ADP-ribose) polymerase. Br J Pharmacol 2004;143:186–92.
[8] Erdelyi K, Bakondi E, Gergely P, Szabo C, Virag L. Pathophysiologic role of oxidative stress-induced poly(ADP-ribose) polymerase-1 activation: focus on cell death and transcriptional regulation. Cell Mol Life Sci 2005;62:751–9.
[9] Zhang C, Yang J, Jennings LK. Attenuation of neointima formation through the inhibition of DNA repair enzyme PARP-1 in balloon-injured rat carotid artery. Am J Physiol Heart Circ Physiol 2004;287:659–66.
[10] Spahr R, Piper HM. Microcarrier cultures of endothelial cells. In: Piper HM, editor. Cell Cultures Techniques in Heart and Vessel Research. Heidelberg, Germany: Springer-Verlag; 1990. p. 220–9.
[11] Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca2+ indicators with greatly improved fluorescence properties. J Biol Chem 1985;260:3440–50.
[12] Homburg S, Visochek L, Moran N, Dantzer F, Priel E, Asculai E, et al. A fast signal-induced activation of poly(ADP-ribose) polymerase: a novel downstream target of phospholipase c. J Cell Biol 2000;150:293–307.
[13] Dantzer F, Nasheuer HP, Vonesch JL, de Murcia G, Menissier-de Murcia J. Functional association of poly(ADP-ribose) polymerase with DNA polymerase alpha-primase complex: a link between DNA strand break detection and DNA replication. Nucleic Acids Res 1998;26:1891–8.
[14] Simbulan-Rosenthal CM, Rosenthal DS, Hilz H, Hickey R, Malkas L, Applegren N, et al. The expression of poly(ADP-ribose) polymerase during differentiation-linked DNA replication reveals that it is a component of the multiprotein DNA replication complex. Biochemistry 1996;35:11622–33.