Philips BlueTouch

Scientific background

PHILIPS

Philips – leading innovation in healthcare solutions

As a pioneering global company and number 1 in Home Healthcare, Philips delivers meaningful people-centric healthcare solutions.

We strive to improve people's quality of life by identifying the barriers to their health and wellbeing and then creating solutions to overcome them.

To do this, we listen to the needs of consumers and healthcare professionals so that we can develop insightful innovations that truly improve people's lives.

The result of years of intensive research, Philips BlueTouch brings innovation and ease of use to the management of back pain.

Delivered simply and easily through the use of a wireless wearable patch, Philips BlueTouch offers relief to patients who suffer from mild to moderate musculoskeletal back pain including neck, shoulders and/or lumbar spinal area. The wireless design provides maximum freedom of movement to enable patients to enjoy everyday activities.

Philips BlueTouch

Abstract

Blue LED light-induced endogenous nitric oxide (NO) production in human skin

Purpose

To study non-enzymatic NO release from NO derivates in an aqueous solution and in human skin irradiated with blue LED light (453 nm).

Method

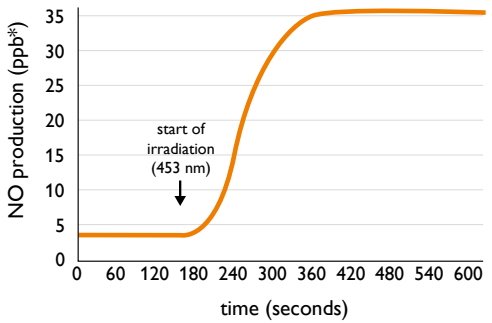
- The release of NO in aqueous nitrite solutions (10 µM NaNO2) induced by blue LED irradiation was investigated at skin relevant pH values (pH 5.5)
- The NO developing in the solution was flushed out with inert gas according to the Kolbe electrolytic method and was determined with a chemiluminescence detector (CLD)

Results

- Using different LED light sources, significantly increased non-enzymatic NO production in nitrite solutions could be determined under conditions existing in human skin (pH 5.5)
- The blue LED light (453 nm)-induced release of NO could be increased by adding $CuCl_2$ and could be inhibited with a specific Cu(I) scavenger

Blue LED light induces the release of NO under skin-relevant conditions

(irradiation of a 10µM nitrite solution with pH 5.5 with blue LED light)



* parts per billion (10^{-9})

adapted from Suschek and Opländer, 2010

Conclusion

Irradiation with blue LED light (453 nm) leads to a significant release of NO from nitrite solutions under skin-relevant conditions.

Background

Human skin contains relatively high concentrations of copper ions and nitrite and has a low pH value of 5.5 to 6, which allows the above-mentioned mechanism of blue LED light-induced NO generation from nitrite to take place. This theory should be verified by intact human skin tests.

Method

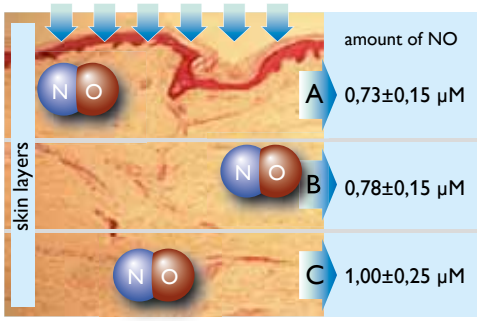
- The skin samples were incubated with Fe^{2+}/DETC, irradiated with blue LED light (453 nm), and frozen in liquid nitrogen
- The skin specimens were subjected to special electron paramagnetic resonance measurements, which demonstrated the presence of NO/Fe/DETC complexes and thereby the generation of NO at different layers

Results

After irradiation with blue LED at a wavelength of 453 nm, increased NO release could be observed, also in deeper skin layers.

Skin irradiation with blue LED light leads to increased NO release, also in deeper skin layers

30 minutes of irradiation with blue LED light (453 nm)



adapted from Suschek and Opländer, 2010

Conclusion

Blue LED light (453 nm)-induced NO release could also be proved in deeper layers of the human skin.

References:
Prof. Dr. C. Suschek, Dr. C. Opländer: Abschlussbericht zum BMBF-Verbundprojekt – „Desinfektion, Entkeimung und biologische Stimulation der menschlichen Haut durch gesundheitsfördernde Licht- und Plasmaquellen." Universitätsklinikum Aachen, 2010.

Abstract

Blue LED light-induced non-enzymatic nitric oxide (NO) production

Purpose

To study non-enzymatic NO release from nitrite and nitrosated proteins by irradiation with blue LED light (453 nm) compared with UVA light (360 nm).

Background

Bioactive NO can also be released non-enzymatically. This occurs through photolysis of nitrite (NO_2) and nitrosated proteins, e.g. S-nitrosoalbumin (SNO Alb), as was already proved by irradiation with UVA light.

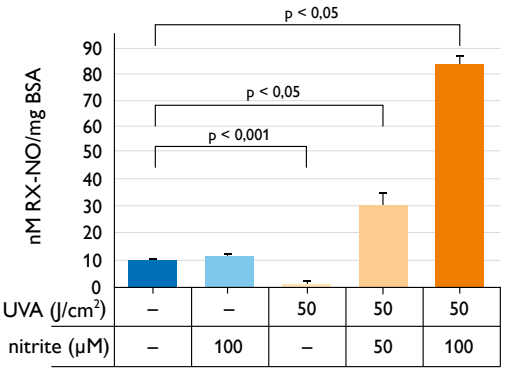
Method

- A serum albumin solution (2% PBS – BSA solution) was examined with and without nitrite (50-100 µM) while irradiated with blue LED light (453 nm) and compared with irradiation with UVA light (360 nm)
- An increase in BSO nitrosation is an indication that the light source used decomposes nitrite photolytically
- A decrease of protein nitrosation in the absence of nitrite during exposure shows that the wavelength used is able to release the NO locked in the nitroso BSA
- The nitrosation of proteins was determined through chemiluminescence detection

Results

- Without adding nitrite, the UVA-treated specimens showed a clear decrease in the amount of nitrosated proteins (positive control)
- By adding different amounts of nitrite, the nitrosation of BSA can be increased by UVA irradiation with the extent of the increase depending on the dose

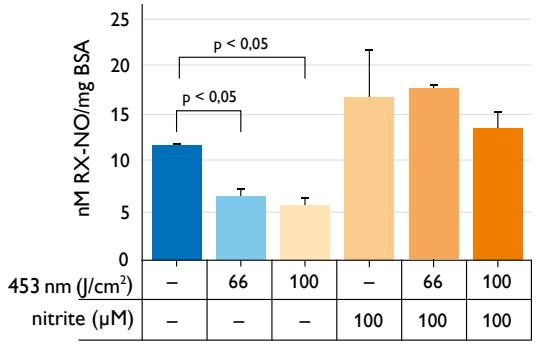
UVA irradiation leads to photolytic decomposition of nitrite and the release of NO from nitrosated proteins



adapted from Liebmann and Born, 2010

- Irradiation with blue LED (453 nm) did not show any increase in nitrosation of BSA in the presence of nitrite
- However, a decrease in nitrosation depending on the irradiation intensity of the blue LED light could be proved

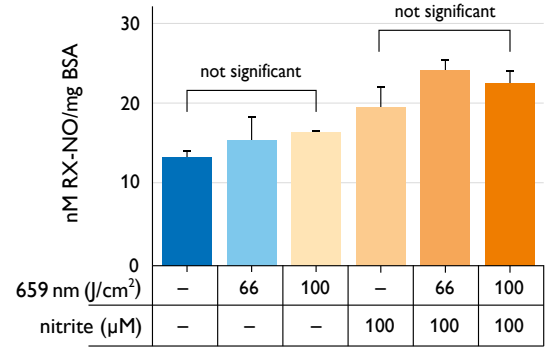
Irradiation with blue LED light results in NO release from nitrosated proteins



adapted from Liebmann and Born, 2010

- A negative control test with light at 658 nm showed no effects

Irradiation with light with a wavelength of 659 nm showed no effects



adapted from Liebmann and Born, 2010

Conclusion

Irradiation with blue LED light (453 nm) causes the release of NO from nitrosated proteins.

References:
Liebmann J, Born M, Kolb-Bachofen MV: "Blue-light irradiation regulates proliferation and differentiation in human skin cells". In: J Invest Dermatol., Vol. 130, pp. 259 – 269, 2010.

Abstract

Blue LED light with a wavelength of 453 nm is not toxic to endothelial cells and keratinocytes

Purpose

To evaluate the biological efficacy of different light sources on keratinocytes and endothelial cells in the human skin with a view to toxicity, proliferation and differentiation behavior.

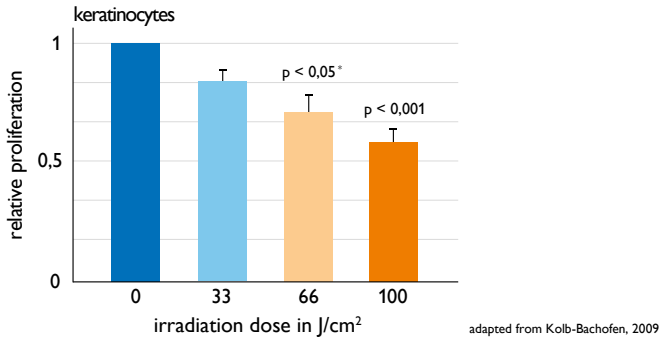
Method

- Keratinocytes for the different experiments were isolated from skin resections and expanded. In addition, human microvascular endothelial cell line (HMEC -1) was used. The cells were cultivated and, after treatment with different parameters, they were analyzed with established molecular biological methods
- To analyze cell proliferation, the AlamarBlue assay or the neutral red uptake assay were used
- To establish a possible toxic effect of the treatments, the cells and cell nuclei were marked with fluorescent dyes and subjected to a microscopic morphological examination
- In addition, the decomposition of aptosis-specific PARP proteins (Poly (ADP Ribose) Polymerase) was demonstrated by means of the western blot technique
- The differentiation degree of human keratinocytes was examined with specific markers (involucrin, keratin[1]) in real-time PCR. The appropriate differentiation markers for endothelial cells are not known

Results

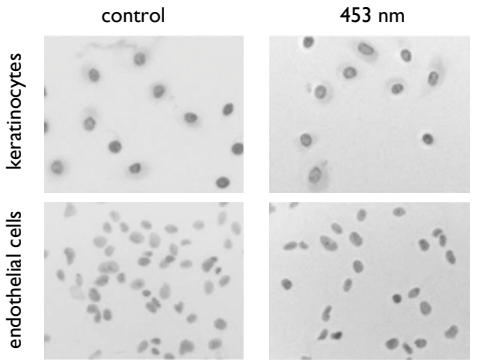
- Irradiation of the cells with LED light at wavelengths of 412, 419 and 426 nm reduced proliferation on account of toxicity. The toxic effects increase at shorter wavelengths and higher doses
- When a blue LED light source with a wavelength of 453 nm was used, a dose-dependent decrease in cell proliferation was also observed
- The measurement of the energy status (ATP content) shows that the energy equivalents do not decrease but significantly increase at an irradiation dose of 100 J/cm²
- Contrary to the shorter wavelengths tested (412 – 426 nm), the proliferation reduction observed at a wavelength of 453 nm cannot be attributed to increased toxicity but, at least in the case of keratinocytes, can be attributed to increased differentiation

Irradiation of skin cells with blue LED light (453 nm) leads to a dose-dependent decrease in proliferation



adapted from Kolb-Bachofen, 2009

Multiple irradiations of skin cells with blue LED light (453 nm) is not toxic

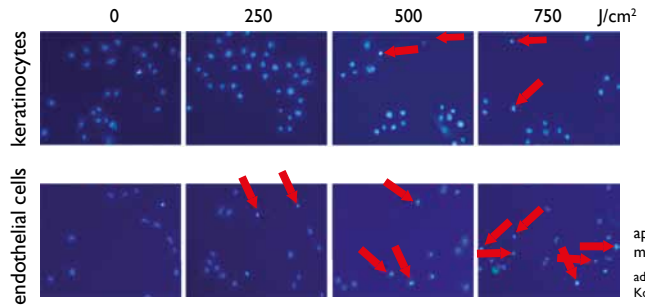


irradiation: 3 times 100 J/cm²

adapted from Kolb-Bachofen, 2009

- Even after three treatments with 100 J/cm² per 24 hours on three consecutive days, the cell cultures showed no signs of aptosis
- A comparative test involving irradiation at all wavelengths and using aptosis marker PARP, which decomposes in case of apoptic processes, confirmed these results
- An investigation into the toxicity limit depending on the irradiation dose showed that blue LED light is not toxic at a dose up to 250 J/cm²
- UVA light (365 nm) is already toxic at an irradiation dose of 30 J/cm²

The irradiation of skin cells with blue LED light (453 nm) is not toxic up to a dose of 250 J/cm²



apoptotic cells are marked with arrows

adapted from Kolb-Bachofen, 2009

Conclusion

Blue LED light with a wavelength of 453 nm is not toxic for skin cells up to an irradiation dose of 250 J/cm².

References:
Prof. Dr. Kolb-Bachofen V: Abschlussbericht zum BMBF-Verbundsprojekt – „Desinfektion, Entkeimung und biologische Stimulation der menschlichen Haut durch gesundheitsfördernde Licht- und Plasmaquellen". Universität Düsseldorf, 2010.

Abstract

The release of nitric oxide (NO) leads to vasodilatation

Background

NO is formed in the endothelial cells of blood vessels and triggers a series of biological processes through cyclic guanosine monophosphate signaling[1]. NO is the only known endogenous radical that works as a signal transmitter[2]. One of the most important effects triggered by NO is vasodilatation through smooth muscle relaxation.

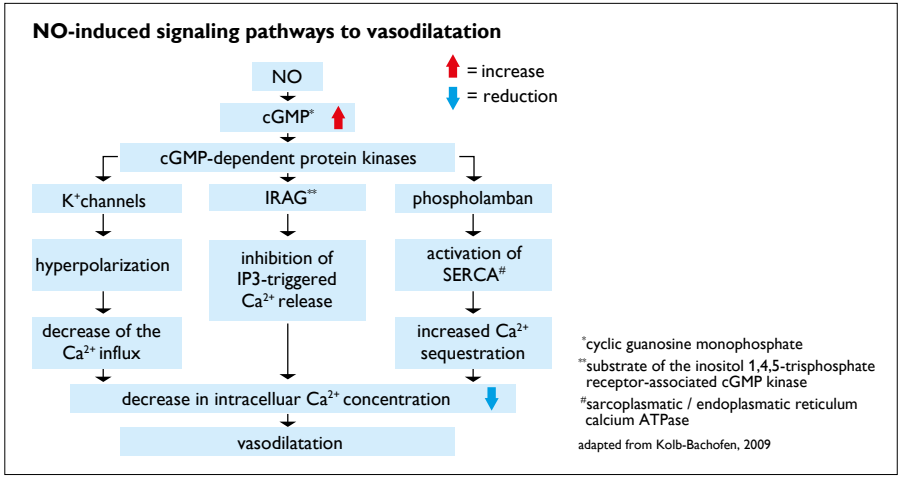
Results

It could be demonstrated that NO activates soluble guanylate cyclase (sGC). NO binding to the heme domain of guanylate cyclase forms a nitrosyl-heme complex. By bonding with the enzyme guanosine triphosphate (GTP) and eliminating a phosphate domain, this configuration converts into the messenger substance cyclic guanosine monophosphate[3]. The intracellular increase in cGMP level triggers the activation of cGMP-dependent protein kinases PKG I and PKG II, with PKG I being mainly responsible for triggering vasodilatation[4,5]. Currently three different signaling pathways are known through which the NO-triggered vasodilatation can take place. All three mechanisms lead to a decrease of the intracellular calcium concentration, which causes relaxation of the flat musculature.

1. The activated PKG I phosphorylates various membrane proteins of the sarcoplasmic reticulum, such as the protein phospholamban[6]. In their dephosphorylated state, phospholamban monomers inhibit the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) by binding the cytoplasmic and membranous domains of the enzyme, thereby causing aggregation of the Ca^{2+} pumps. Phosphorylated phospholamban releases the previously bonded SERCA and activates it[7]. This leads to rapid sequestering of intracellular calcium, which again reduces the influx of extracellular calcium into the sarcoplasmic reticulum[8].

2. The activated PKG I also phosphorylates the substrate of inositol 1,4,5-trisphosphate receptor-associated cGMP kinase (IRAG)[9]. The phosphorylating of IRAG leads to a strong inhibition of IP3-triggered Ca^{2+} release from the sarcoplasmic reticulum.

3. Moreover, NO can phosphorylate and activate through the PKG/Ca^{2+}- dependent potassium channels[10, 11]. The resulting hyperpolarization of the cell membrane reduces the effectiveness of the depolarization signal, thereby triggering vasodilatation.



- Reduction of intracellular calcium concentrations decreases the formation of Ca^{2+}-calmodulin-dependent myosin light chain kinase complex. It reduces the phosphorylation of myosins, thereby inhibiting vasoconstriction[12].

Conclusion

NO-induced signaling pathways lead to a reduction of the intracellular calcium concentration in smooth muscle cells and cause vasodilatation by relaxing the vascular smooth muscles.

References:
1. Gewaltig MT, Kojda G: "Vasoprotection by nitric oxide: mechanisms and therapeutic potential". In: Cardiovasc Res Vol. 55, pp. 250 – 260, 2002.
2. Lucas KA, Pitari GM, Kazerounian S, Ruiz-Stewart I, Park J, Schulz S, Chepenik KP, Waldman SA: "Guanylyl cyclases and signaling by cyclic GMP". In: Pharmacol Rev, Vol. 52, pp. 375 – 414, 2000.
3. Ignarro LJ, Cirino G, Casini A, Napoli C: "Nitric oxide as a signaling molecule in the vascular system: an overview". In: J Cardiovasc Pharmacol, Vol. 34, pp. 879 – 886, 1999.
4. Pfeifer A, Klatt P, Massberg S, Ny L, Sausbier M, Hirneiss C, Wang GX, Korth M, Aszódi A, Anderson KE, Krombach F, Mayerhofer A, Ruth P, Fässler R, Hofmann F: "Defective smooth muscle regulation in cGMP kinase I-deficient mice". In: EMBO J, Vol. 17, pp. 3045 – 3051, 1998.
5. Massberg S, Sausbier M, Klatt P, Bauer M, Pfeifer A, Siess W, Fässler R, Ruth P, Krombach F, Hofmann F: "Increased adhesion and aggregation of platelets lacking cyclic guanosine 39,59-monophosphate kinase I". In: J Exp Med, Vol. 189, pp. 1255 – 1263, 1999.
6. Cornwell TL, Pryzwansky KB, Wyatt TA, Lincoln TM: "Regulation of sarcoplasmic reticulum protein phosphorylation by localized disease. cyclic GMP-dependent protein kinase in vascular smooth muscle cells". In: Mol Pharmacol, Vol. 40, pp. 923 – 931, 1991.
7. Simmermann HK, Jones LR: "Phospholamban: protein structure, mechanism of action, and role in cardiac function". In: Physiol Rev, Vol. 78, pp. 921– 947, 1998.
8. Cohen RA, Weisbrod RM, Gericke M, Yaghoubi M, Bierl C, Bolotinav M: "Mechanism of nitric oxide-induced vasodilatation – Refilling of intracellular stores by sarcoplasmic reticulum Ca2+ ATPase and inhibition of store-operated Ca influx". In: Circ Res, Vol. 84, pp. 210 – 219, 1999.
9. Schlossmann J, Ammendola A, Ashman K, Zong X, Huber A, Neubauer G, Wang GX, Allescher HD: "Regulation of intracellular calcium by a signalling complex of IRAG, IP3 receptor and cGMP kinase Ib." In: Nature, Vol. 404, pp. 197– 201, 2000.
10. Bolotina VM, Najibi S, Palacino JJ, Pagano PJ, Cohen RA: "Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle". In: Nature, Vol. 368, pp. 850 – 853, 1994.
11. Sausbier M, Schubert R, Voigt V, Hirneiss C, Pfeifer A, Korth M, Kleppisch T, Ruth P, Hofmann F: "Mechanisms of NO / cGMP-dependent vasorelaxation." In: Circ Res, Vol. 87, pp. 825 – 830, 2000.
12. Horowitz A, Menice CB, Laporte R, Morgan KG: "Mechanisms of smooth muscle contraction". In: Physiol Rev, Vol. 76, pp. 967 – 1003, 1996.

Abstract

The release of nitric oxide (NO) in the skin leads to a systemic increase in blood flow rates and a decrease in blood pressure

Purpose

To investigate to what extent whole body UVA irradiation influences the blood pressure in healthy test subjects through continuous NO release.

Background

Human skin contains photolabile NO derivates such as nitrite and S-nitrosated composites, which decompose as a result of UVA irradiation and release vasoactive NO.

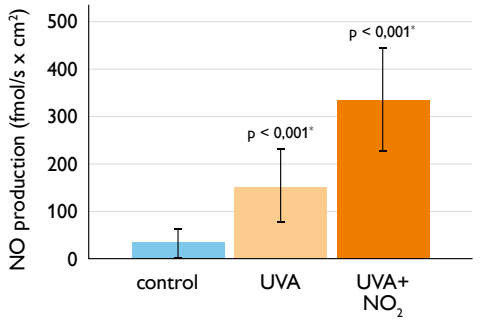
Method

- An airtight chamber (16 cm^2) with a UVA-transparent window was placed on the forearms of the test persons to collect the gaseous NO released from the skin. The NO and the nitrosated proteins formed after irradiation were quantified by chemiluminescence detection
- To determine the biological activity of the blood plasma of non-irradiated and UVA-irradiated (20 J/cm^2, 30 min.) test subjects, the formation of cyclic guanosine monophosphates (cGMP) was determined in rat lung fibroblasts (RFL6 cells). In the presence of superoxide dismutase (500 U/ml) and isobutylmethylxanthin (0.6 mmol/l), the RFL6 cells (3x105) were incubated with the blood plasma. In a further experiment, the RFL6 cells were additionally incubated with NO radical scavenger cPTIO
- For the in-vivo determination of intradermal NO release or the forming of nitrosated proteins, test subjects were irradiated with UVA light (20 J/cm^2) for 30 or 15 minutes. The control test subjects were not irradiated
- In a further in-vivo experiment, healthy test subjects were treated with a cream containing isotope-marked nitrite (100 µmol in 20 ml) and then received a whole-body irradiation with UVA light (20 J/cm^2). The amounts of ^{15}N-marked S-nitrosated compounds were determined by cavity leak-out spectroscopy

Results

- The gaseous NO from the skin increased four times through UVA irradiation compared to the non-irradiated control group
- Applying nitrite-containing skin cream prior to irradiation increased the formation of photo-induced NOs significantly again

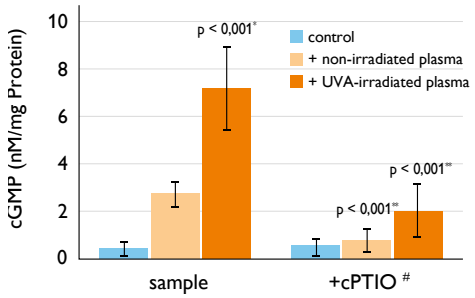
UVA irradiation of the skin leads to release of NO



*p-value compared to control
adapted from Opländer et al. 2009

- The UVA irradiation also leads to a significantly increased formation of cGMP. This increase could be significantly reduced by administering an NO radical scavenger (cPTIO)

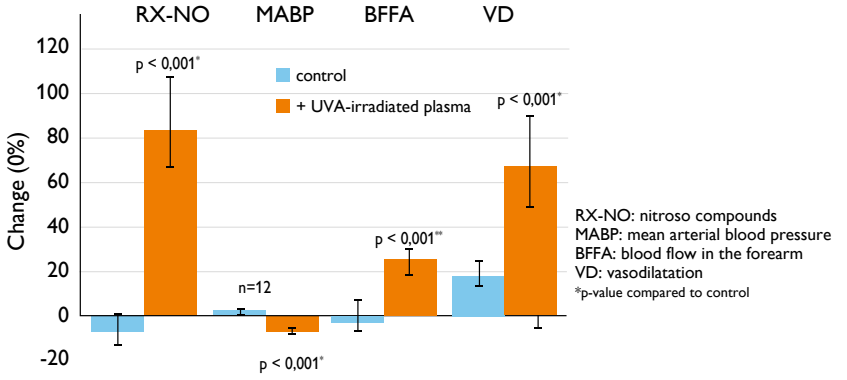
The NO released by UVA irradiation strengthens the formation of cGMP



*p-value compared to the sample that has not been treated with cPTIO;
#1H-imidazole-1-yloxy-2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-3-oxide
adapted from Opländer et al. 2009

- In in-vivo experiments, an increase in S-nitrosated compounds in the skin, such as s-nitroso albumin, could be established
- After application of a cream with ^{15}N-nitrite in healthy test subjects, UVA irradiation resulted in a systemic increase of ^{15}N-marked S-nitroso compounds
- This increase correlated with a significant decrease of systolic and diastolic blood pressure, which lasted up to 90 minutes after a 15-minute irradiation
- The increased plasma concentration of nitrosated compounds also correlated with a systemic increase of the blood flow rate and vasodilatation

NO production induced by UVA light leads to an increase of systemic blood flow and to a decrease of blood pressure



RX-NO: nitroso compounds
MABP: mean arterial blood pressure
BFFA: blood flow in the forearm
VD: vasodilatation
*p-value compared to control

Conclusion

Irradiation with UVA light leads to a systemic increase of S-nitroso compounds and increased release of NO. This correlates with the increased formation of cGMP, vasodilatation, systemic increase of the blood flow rate and a decrease of blood pressure.

References:
Opländer C, Volkmar C, Paunel-Görgülü A, van Faassen E, Heiss C, Kelm M, Halmer D, Mürtz M, Pallua N, Suschek C: "Whole body UVA irradiation lowers systemic blood pressure by release of nitric oxide from intracutaneous photolabile nitric oxide derivates". In: Circ. Res., Vol. 105, pp. 1031 – 1040, 2009.

Abstract

Nitric oxide (NO) regulates the release of substance P in spinal cord synaptosomes

Purpose

To investigate to what extent NO directly affects the nerve endings and regulates synaptic transmission.

Background

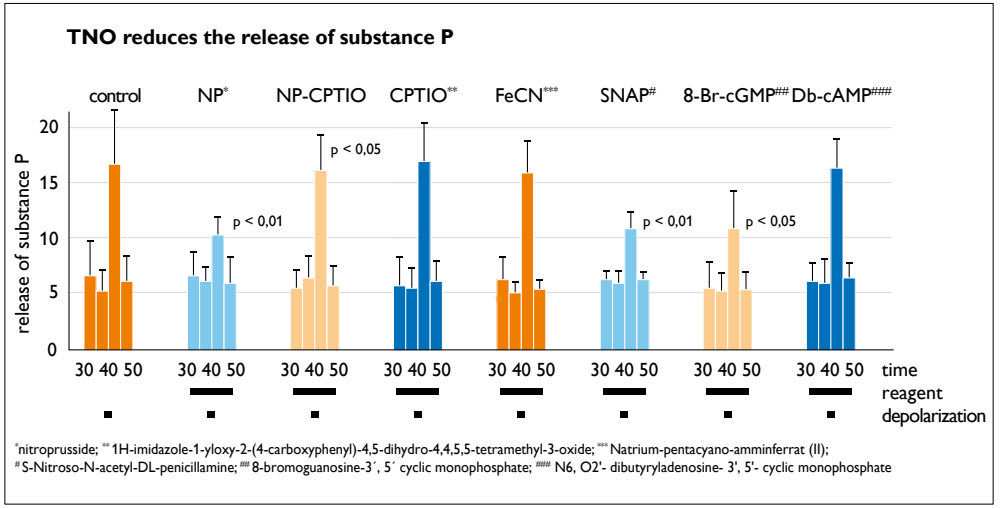
The neuropeptide substance P plays an important role in the regulation of multiple physiological processes. Activation of the pain receptors is one of the factors triggering the release of substance P and this substance is also involved in the transmission of pain.

Method

- Rat spinal cord was used to isolate synaptosomes. The rat spinal cord was homogenized and centrifugated and resuspended. The protein content was determined by means of the method of Lowry et al. (1951)
- Next, the synaptosomes were superfused. 40 minutes after the superfusion started, the synaptosomes were depolarized with a high concentration of KCL (30 mM)
- Different reagents (100 µM in each case) were added to the standard perfusion medium (Krebs-Ringer buffer) 10 minutes before depolarization and their effects were investigated
- The reagents were NO donor sodium nitroprusside (SNP), NO radical scavenger 1H-imidazol-1-yloxy-2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-3-oxide (cPTIO), cyanide ion donor sodium pentacyanoammineferrate (II) (FeCN), NO donor S-nitroso-N-acetyl-DL-penicillamine (SNAP) and two cyclic nucleotide derivates: N6,O2'-dibutyryl adenosine 3':5'-cyclic monophosphate (Db-cAMP) and 8-bromoguanosine 3',5'-cyclic monophosphate (8-Br-cGMP)
- The concentration of substance P was determined with an EIA (enzyme immunoassay) kit. Here the antiserum identified the C-terminal sequencing of the peptides. The final absorption power was measured with a microplate reader
- The concentration of cGMP was also determined with an EIA kit
- The release of substance P was indicated by synaptosomes in femtomols per milligram protein. The basal release was defined as average quantity in two 5-minute fractions, which were taken immediately after depolarization (a 30 to 35 and a 35 to 40-minute fraction after start of perfusion)

Results

- Compared with the basal amounts, a 2.7 times higher release of substance P was observed by depolarizing the synaptosomes (control)
- This induced release of substance P was reduced to 60 percent by administering NO donor sodium nitroprusside (100 µM). This effect depended on the NP concentration
- To confirm that the reduction of substance P release was attributable to the increased availability of NO, the experiment was repeated with NO donor (SNAP). Here similar results were obtained (55% reduction)
- In a further experiment, the NP-induced reduction of substance P release could be significantly decreased by adding NO radical scavenger (cPTIO)
- The addition of two cyclic nucleotide derivates showed that membrane permeable cGMP also leads to a significant reduction of the depolarization-induced release of substance P. No effect could be observed with cyclic AMP
- It could also be observed that the NO amount released by sodium nitroprusside sufficed to activate the guanylate cyclase and was enough to increase the cGMP amount in the synaptosomes. NP brings about a significant increase of the cGMP amounts depending on the NP concentration. The concentration dependencies observed coincide with those that were observed for the NP-induced reduction of substance P release
- The time after which an increased cGMP amount could be observed after NP administration coincides with the time after which the effect of NP on substance P release could be shown



Conclusion

NO immediately affects the nerve endings and reduces the pain transmission by increasing the formation of cGMP and thereby reducing the release of substance P.

References:
Kamisaki Y, Nakamoto K, Wada K, Itoh T: "Nitric oxide regulates substance P release from rat spinal cord synaptosomes". In: J. Neurochem., Vol. 65, pp. 2050 – 2056,1995

Abstract

Nitric oxide (NO) reduces the development of inflammatory processes

Purpose

To investigate the effects of NO on the migration of neutrophil granulocytes in inflammatory processes.

Background

Neutrophil granulocytes circulate in blood and migrate into inflamed tissue. This is a process facilitated by adhesion molecules and chemokines. Four steps can be identified in the recruitment of neutrophil granulocytes to the center of an infection: selectin-facilitated rolling along the vessel endothelium, chemokine-facilitated increase in integrin affinity, stable integrin-facilitated adhesion to the endothelium and migration through the endothelial tissue.

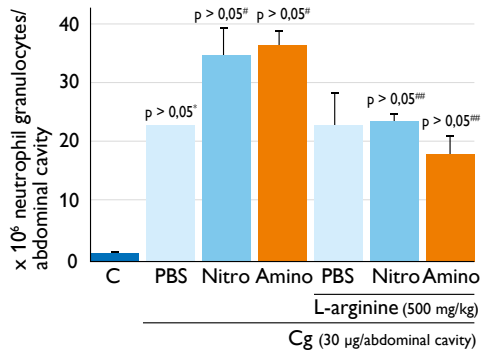
Method

- For all experiments rats and mice were injected with a phosphate-buffered saline solution (PBS), a selective NO-synthase inhibitor aminoguanidine (Amino) or a non-selective NO-synthase inhibitor NG-nitro-L-arginine (Nitro). After 30 minutes the endotoxin carragene (Cg) was administered intraperitoneally
- For the in-vivo determination of the migration of neutrophil granulocytes, the rats and mice were killed and cells were taken from the abdominal cavity. The results were represented as the number of neutrophil granulocytes per abdominal cavity
- Leukocyte rolling and adhesion were evaluated by microscopic examination of the mesenteric tissue. This involved an analysis of the interaction of the leukocytes with the luminal surface of the venulial epithelium
- The apoptotic index of the neutrophil granulocytes harvested from the abdominal cavity was established

Results

- The administration of both NO synthase inhibitors significantly increases the migration of neutrophil granulocytes to the induced inflammatory reaction
- It may be assumed that the L-arginine/NOS pathway is involved in this process

NO reduces the migration of neutrophil granulocytes in inflammations

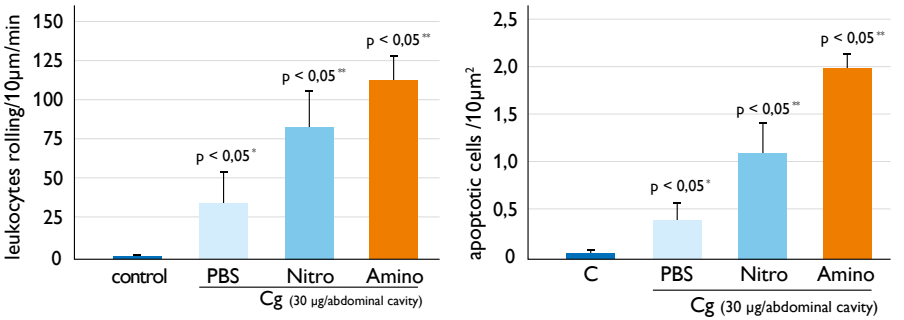


PBS: phosphate-buffered saline solution; Nitro: N^G-N-nitro-L-arginine; amino: aminoguanidine; Cg: carrageen *p-value compared to control; #p-value compared to PBS-treated, Cg-injected, sample; ###p-value compared to respective group, treated with Nitro or Amino

adapted from Dal Secco et al. 2004

- It was established that the NOS inhibition strengthens the neutrophil migration by increasing the inflammation-induced rolling and adhesion of neutrophil granulocytes to venous endothelium

NO reduces the interaction of neutrophil granulocytes with endothelial cells

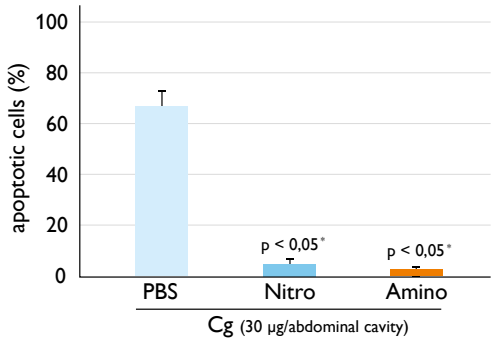


PBS: phosphate-buffered saline solution; Nitro: N^G-nitro-L-arginine; Amino: aminoguanidine; Cg: carrageen *p-value compared to control; **p-value compared to PBS-treated, Cg-injected, sample

adapted from Dal Secco et al. 2004

- It was shown that the increased migration of the neutrophil granulocytes as a result of NOS inhibition coincides with a significant decrease of the apoptosis rates of these cells

NO increases the apoptosis rates of activated neutrophil granulocytes



PBS: phosphate-buffered saline solution; Nitro: N^G-nitro-L-arginine; p-value compared to PBS-treated, Cg-injected, sample

adapted from Dal Secco et al. 2004

Conclusion

In inflammatory rea ads to a decrease of the migration of neutrophil granulocytes and induces their apoptosis.

References:
Dal Secco D, Paron JA, de Oliveira SH, Ferreira SH, Silva JS, Cunha F de Q: "Neutrophil migration in inflammation: nitric oxide inhibits rolling, adhesion and induces apoptosis." In: Nitric Oxide, Vol. 9, pp.153 – 164, 2003.

Abstract

The antioxidative effects of nitric oxide (NO) protect against cell damage and cell death, also in the central nervous system

Purpose

To investigate the antioxidative effects of NO and the protective influence on the central nervous system[1].

Background

In vivo, NO develops in endothelial cells, astroglias and some neurons[2,3]. By activating the guanylate cyclases and increasing the formation of cGMP, NO regulates cell-to-cell communication[4] and the cerebral blood flow[5]. Because of its chemical composition – a weak, nitrogen-centered free radical – NO could show antioxidative effects, which protect against, amongst other things, nerve damage. Several in-vitro and in-vivo studies support this hypothesis[6] and show that NO is a strong antioxidative agent that protects dopaminergic neurons against reactive oxygen species and thereby against oxidative stress[7, 8, 9].

Results

Forming of reactive oxygen species in the central nervous system

- Free radicals are the inevitable byproducts of all electron-transmitted redox reactions, such as the various processes of the respiratory chain[1]
- It is suspected that free radicals such as • OH induce neurodegenerative pathways and develop as a result of ischemia and reperfusion-conditioned processes[10]

Neurobiology of NO

- Compared with the highly toxic hydroxyl radicals[11,12] the NO radicals are several orders of magnitude less reactive and thereby lead to opposite neurobiological processes[1]
- The effects of NO last long and NO can work over relatively long distances[1]

Antioxidative and neuroprotective properties of NO

- NO can protect against hydroxyl radical-induced DNA damage and against H_2O_2-induced cell death and has a large capacity to end radical-induced lipid peroxidation as vitamin E[13,14]
- Even in low concentrations, NO can modulate important components of the antioxidative protection, the glutathione metabolism for instance, by inducing an increase in the expression of enzymes that are key to the glutathione synthesis: g-glutamylcysteine synthetase (gGCS) and g-glutamyl transpeptidase[15]
- NO is a decisive factor in regulating the activity of heme oxygenase 1. The NO-induced increase in activity of the enzyme leads to a high resistance of the endothelium against oxidative stress[16]
- When enzymes containing iron and thiol groups are nitrosylated by NO, this significantly changes their biological activity in the central nervous system. Redox reactions and the formation of reactive oxygen species are reduced, resulting in a decrease of oxidative stress[8,9]. NO also deactivates cysteine protease-induced apoptosis and neurotoxicity[1]
- It was also shown that NO regulates the antioxidative thioredoxin system and the antiapoptic Bcl-2 protein through a cGMP-dependent mechanism, thereby providing protection against nerve degeneration[16]
- Based on in-vivo and in-vitro cerebral preparations, it was also proved that NO acts as a free radical scavenger[7, 8]. NO as a weak radical with a relatively long half-life period eliminates highly reactive free radicals with a shorter half-life period, such as superoxide anion radicals (• O_2^-), hydroxyl radicals (• OH), lipid peroxyl radicals (LOO •) and thiyl radicals (GS •), which are formed during oxidative stress. In the same process, nitrogen species such as nitrite (NO_2^-), nitrate (NO_3^-), S-nitrosoglutathione (GSNO) and peroxynitrite ($ONOO^-$) are formed[5, 6, 17, 18, 19]
- The nitrites and nitrates that act as NO donors can be quickly eliminated by anion transporters in the brain, minimizing their cytotoxicity in-vivo
- GSNO is 100 times more effective than the endogenous antioxidant glutathione (GSH) and is therefore able to completely block the peroxidative effect of $ONOO^-$. GSNO also inhibits formation of •OH and lipid peroxidation in the brain induced by ferrous citrate complexes[7-9] and protects dopaminergic cerebral neurons against damage[20,21]. These effects of GSNO are the result of the release of NO
- GSNO is an important part of the antioxidative GSH system. As GSH is formed in the astroglia and cannot be transported into cerebral neurons, GSNO serves as an appropriate carrier substance

Reactions of unpaired electrons: • NO eliminates lipid, oxygen and thiol radicals

I.	LOO •	+ • NO	⟶ LOONO	
II.	O2 • –	+ • NO	⟶ [ONOO–]	⟶ NO3–
III.	• OH	+ • NO	⟶ [HONO]	⟶ NO2–
IV.	GS •	+ • NO	⟶ GSNO	
V.	Peptide cis	+ • NO	⟶ Peptide cisNO	

adapted from Chiueh, 1999

Conclusion

The antioxidative effects of NO protect cells against DNA damage, the oxidation of proteins and lipid peroxidation, also in neurons and brain cells.

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Abstract

The anti-inflammatory effects of nitric oxide (NO) can protect against cell damage and cell death

Purpose

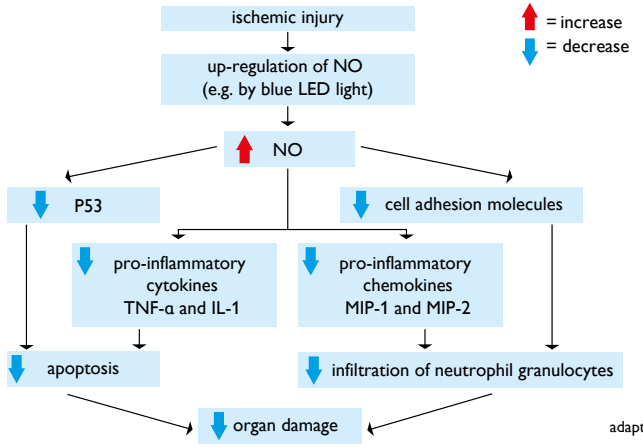
To investigate the effect of NO on ischemia and reperfusion-conditioned damage[1].

Background

Thanks to its antiapoptotic and anti-inflammatory properties, NO can have a protective effect. NO also influences the cell's signaling pathways and modulates gene expression through inhibition of gene expression-regulating core proteins[1].

Results

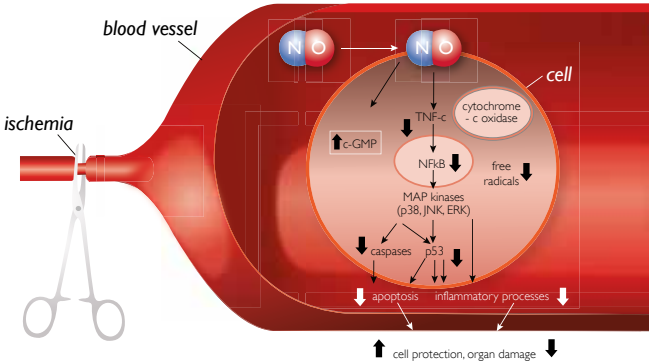
Regulation by NO can have the following molecular effects:



adapted from Philips et al., 2009

- Ischemic damage results in a decrease of NO production, which can be compensated by the exogenous administration of NO or the stimulation of endogenous NO formation
- NO protects against inflammation-conditioned cell damage and cell death• NO causes inhibition of cytochrome-c-oxidase in the mitochondria, resulting in the formation of fewer free radicals[2, 3]
- TNF-α stimulates the infiltration of neutrophil granulocytes during reperfusion[4]. TNF-α also induces the synthesis of inflammation-promoting chemokines in ischemic tissue. In addition, it activates transcription factor NF-kB, the gene regulating the inflammation response[5]. The level of the pro-inflammatory cytokine TNF-α is reduced by NO[6, 7]
- NO itself also inhibits the formation of NF- kB[8]
- NO is an important regulator of mitogen-activated protein kinases (MAPKs), which modulate gene and protein expression and thereby influence the signaling pathways of cell growth, differentiation and apoptosis.[9] They were activated by early ischemic processes[10]
- Reduction of NF-kB leads to inhibition of various MAP kinases, such as p38, ERK (extracellular signal-related kinases) and JNK (c-jun-n-terminal kinase)[1]
- NO is also shown to have direct effect on ERK1/2 and suppresses their activation[11]
- NO also inhibits caspases, which is a family of cysteine proteases that play an important role in the initiation and actual implementation of apoptosis[12]
- The decrease of p38 also leads to inhibition of caspase-3 and inhibits p53 signaling[1]
- Reduction of p53, ERK and JNK can decrease inflammatory processes. The inhibition of caspases and p53 again results in reduced apoptosis[1]

NO influences cell signaling pathways and inhibits gene expression-regulating core proteins



adapted from Philips et al., 2009

Conclusion

In case of ischemia/reperfusion, the anti-inflammatory and antiapoptotic effects of NO help to protect the cells, reducing organ damage.

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Abstract

Nitric oxide (NO) plays a decisive role in the repair of injured skeletal muscles

Background

The healing process of skeletal muscles consists of three phases[1]:

1. Destruction: degeneration of muscle fibers and inflammation
2. Repair: phagocytosis of necrotic fibers. Regeneration of muscle fibers, forming of cicatricial tissue
3. Reconstruction: ripening of regenerated muscle fibers, contraction and readjustment of the cicatricial tissue, restoration of the functional performance of the muscle

NO appears to be an important regulatory factor in all phases of muscle repair[1]

Results

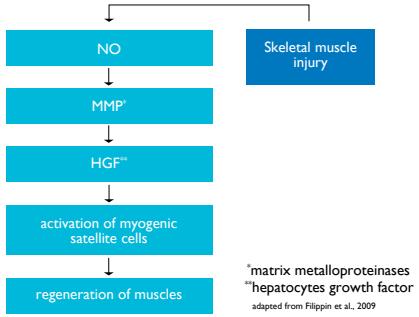
Effects of NO in the degenerative phase:

- During the inflammatory phase of a muscle injury, the local NO production increases[2, 3]
- The NO released in the muscles reduces the cell damage due to inflammation by stimulating the apoptosis of inflammation-promoting cells and reducing the expression of cell adhesion molecules[4]
- NO also reduces the lysis of muscle cells induced by neutrophils and reduces the formation of reactive oxygen species[4]

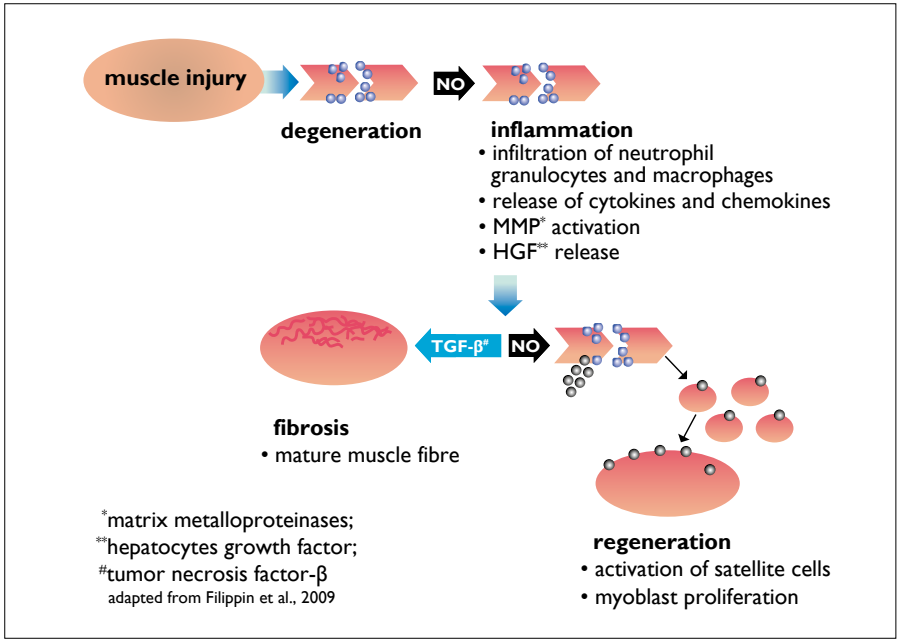
Effects of NO in the regeneration and reconstruction phases:

- The formation of new blood vessels is an essential process in muscle regeneration. Angiogenesis is a precondition for the healing of injured muscles because it will restore the blood vessels, the blood flow and the oxygen supply to the tissue. NO also plays a decisive role in angiogenesis, as it acts as vasodilator and activates various growth factors[5]
- NO regulates the expression of many genes that help repair localized damage, especially the many members of the matrix-metalloproteinase (MMP) family[6]
- The release of growth factor HGF as a result of muscle stretches depends on the presence of NO[7]
- NO activates MMPs that also trigger the release of growth factor HGF and thereby activates the satellite cells in the muscle

NO stimulates the repair and growth of damaged muscle fibres



- NO and TGF-β appear to be opposites. The inhibition of the NO synthesis strongly increases the amount of TGF-β and stimulates the formation of cicatricial tissue[9]. The regulation of the induction of regenerative or fibrotic processes also appears to depend on the balance between the amount of NO and that of TGF-β[1]



Conclusion

NO is an important regulator in all three phases of healing injured skeletal muscles.
NO modulates inflammatory processes, stimulates regeneration and counteracts fibrosis.

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