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Role of nitric oxide in optic nerve head blood flow regulation during an experimental increase in intraocular pressure in healthy humans

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ABSTRACT

The present study set out to investigate whether nitric oxide, a potent vasodilator, is involved in the regulatory processes in optic nerve head blood flow during an experimental increase in intraocular pressure (IOP). The study was conducted in a randomized, double-masked, placebo-controlled, three way cross-over design. 12 healthy subjects were scheduled to receive either L-NMMA (an unspecific nitric oxide synthase inhibitor), phenylephrine (an α-adrenoceptor agonist) or placebo on three different study days. Optic nerve head blood flow was measured using laser Doppler flowmetry and IOP was increased stepwise with a suction cup. Mean arterial pressure (MAP) and IOP were measured non-invasively and ocular perfusion pressure (OPP) was calculated as OPP = 2/3 MAP-IOP. Administration of L-NMMA and phenylephrine significantly increased MAP and therefore OPP at rest ($p < 0.01$). L-NMMA significantly reduced baseline blood flow in the optic nerve head ($p < 0.01$). Application of the suction cup induced a significant increase in IOP and a decrease in OPP ($p < 0.01$). During the stepwise increase in IOP, some autoregulatory potential was observed until OPP decreased approximately −30% below baseline. None of the administered substances had an effect on this autoregulatory behavior ($p = 0.49$). The results of the present study confirm that the human optic nerve head shows some regulatory capacity during a decrease in OPP. Nitric oxide is involved in the regulation of basal vascular tone in the optic nerve head but does not seem to be involved in the regulatory mechanisms during an acute increase in IOP in young healthy subjects.

1. Introduction

Glaucoma, one of the leading causes of blindness in the industrialized world, is associated with disturbances in ocular blood flow autoregulation (Schmidl et al., 2011a). The term blood flow autoregulation describes the ability of a vascular bed to keep its blood flow stable, despite variations in perfusion pressure. In healthy subjects it has been shown that a decrease in ocular perfusion pressure (OPP) does not lead to a parallel decrease in choroidal and optic nerve head blood flow (Pillunat et al., 1997; Polska et al., 2007; Riva et al., 1997a,b; Schmidl et al., 2012; Schmidl et al., 2011b; Simader et al., 2009; Weigert et al., 2005). This is explained by the presence of a regulatory capacity of the vascular beds, meaning that blood flow does not decrease despite a decline in OPP (Schmidl et al., 2011a). However, when OPP decreases below the regulatory range, blood flow starts to decrease linearly. Evidence for this behavior comes from human studies as mentioned above, but has also been observed in rabbits, monkeys, cats and rats (Geijer and Bill, 1979; Kiel and Shepherd, 1992; Kiel and van Heuven, 1995; Piper et al., 2013; Riva et al., 1996; Schmidl et al., 2011a; Zhi et al., 2012).

In patients with glaucoma, reduced OPP has been identified as a risk factor for the onset and progression of the disease

Abbreviation: GON, glaucomatous optic neuropathy; ONH, optic nerve head; LDF, laser Doppler flowmetry; ONHBF, optic nerve head blood flow; NO, nitric oxide; OPP, ocular perfusion pressure; NOS, nitric oxide synthase.

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(Cherecheanu et al., 2013; Leske, 2009). While in primary open angle glaucoma the OPP decrease is caused either by increased intraocular pressure (IOP) or systemic arterial hypotension, the latter causes low OPP in normal tension glaucoma (De Moraes et al., 2012; Grover and Budenz, 2011; Okumura et al., 2012; Topouzis et al., 2013). Further, short-term fluctuations in OPP have been found to be a risk factor for the disease (Mroczkowska et al., 2013; Quaranta et al., 2013; Sung et al., 2009).

These results in combination with findings that ocular blood flow is often reduced in patients with glaucoma support the concept of impaired blood flow autoregulation in glaucoma (Fuchsjager-Mayrl et al., 2004; Hafez et al., 2003; Yanagi et al., 2011). Indeed, blood flow autoregulation has been found to be abnormal in patients with glaucoma (Cherecheanu et al., 2013; Feke and Pasquale, 2008; Kochkorov et al., 2010; Portmann et al., 2011; Schmidl et al., 2011a). Especially, optic nerve head blood flow (ONHBF) seems to be important, since the optic nerve head (ONH) is most likely the location of the primary insult in glaucomatous optic neuropathy (GON) (Malik et al., 2012; Venkataraman et al., 2010).

The Nitric Oxide/Endothelin system has been proposed as a possible target to improve blood flow regulation in glaucoma, because it is an important regulator of vascular endothelial tone in the ocular circulation (Haefliger et al., 2001). For the choroid it has been shown that both substances are involved in blood flow regulation during an experimental increase in OPP in healthy subjects (Fuchsjager-Mayrl et al., 2003; Luksch et al., 2003). In the ONH, administration of a nitric oxide synthase (NOS) inhibitor reduced baseline ONHBF, but did not alter the autoregulatory behavior during isometric exercise (Schmidl et al., 2013). In contrast, blockade of the Endothelin$_A$ receptor had no effect on baseline ONHBF but significantly altered the response of ONHBF to isometric exercise (Boltz et al., 2013). In the present study, we set out to investigate whether NOS inhibition has an effect on ONHBF blood flow regulation during an experimental increase in IOP in healthy subjects. This was done in an effort to gain more insight in the process underlying ONHBF regulation during a decrease in OPP.

2. Material and methods

2.1. Subjects

The present study was performed in adherence to the Declaration of Helsinki and the Good Clinical Practice (GCP) guidelines of the European Union. The study protocol was approved by the Ethics Committee of the Medical University of Vienna. After written informed consent was obtained, 12 healthy female and male subjects completed the present study (age: 25.7 ± 3.6 years, mean ± SD). The sample size calculation was based on unpublished results from previous measurements of ONHBF during an increase in IOP in our laboratory. A repeated measures ANOVA model was underlying this sample size calculation. These data were used for the sample size calculation selecting an α-error of 0.05 and a power of 0.80 and aiming to measure a difference between treatment arms of 10%. Changes in ONHBF below 10% were assumed to be irrelevant.

During the four weeks before the first study day, a screening examination was performed which included recording of medical history and a physical examination, 12-lead electrocardiogram, and assessment of laboratory values (hemoglobin, hematocrit, RBC, MCH, WBC, platelet count, aPTT, thrombin time, sodium, potassium, creatinine, GPT (ALAT), γ-GT, total bilirubin, total protein, Hepatitis B, C and HIV-Serology and urine analysis). In addition, a full ophthalmologic examination was performed. If any clinically relevant abnormality was found as part of the screening examination the subject was not included. In addition, only subjects with ametropia of less than 1 diopter and no evidence of eye disease were recruited to participate in the trial. In the three weeks before the first study day and during the course of the study no intake of concomitant medication was allowed, except oral contraceptives. During the week after completion of the study, a follow up safety investigation similar to the pretreatment examination was performed.

2.2. Drugs and drug administration

NG-monomethyl-L-arginine (L-NMMA, Bachem Distribution Services GmbH, Weil am Rhein, Germany): A bolus of 6 mg/kg over 5 min followed by a continuous infusion of 60 μg/kg/min over 15 min (Schmidl et al., 2013) was applied.

Phenylephrine (Neosynephrine®, Winthrop Breon Laboratories New York, NY, USA): 1 μg/kg/min was administered over an infusion period of 20 min. Since administration of L-NMMA leads to a slight increase in systemic blood pressure at rest (Campbell et al., 2011; Haynes et al., 1993; Mayer et al., 1999), phenylephrine was used as control so that subjects would start at similar OPPs. Phenylephrine has no effect on ocular hemodynamics and in the administered dose has similar effects on systemic blood pressure (Luksch et al., 2003; Schmetterer et al., 1996; Simader et al., 2009).

Physiological saline solution was used as placebo.

A bolus over 5 min followed by a continuous infusion over 15 min of phenylephrine or placebo were prepared and infused subsequently to allow for double-masked conditions.

2.3. Methods

2.3.1. Noninvasive measurement of systemic hemodynamics and OPP

An automated oscillometric device was used for measurement of systolic, diastolic and mean arterial pressure (SBP, DBP, MAP) on the upper arm. Pulse rate (PR) was automatically recorded from a finger pulse-oxymetric device (HP-CMS patient monitor, Hewlett Packard, Palo Alto, CA, USA). Ocular perfusion pressure (OPP) was estimated 2/3 MAP − IOP (Robinson et al., 1986).

2.3.2. Laser Doppler flowmetry (LDF)

Measurement of ONH blood flow was performed using a laser Doppler flowmeter with fundus view (Riva et al., 2010, 1992). With this device the vascularized tissue is illuminated by coherent laser light. Scattering on moving red blood cells (RBCs) leads to a frequency shift in the scattered light. In contrast static components in tissue do not change light frequency, but lead to randomization of light directions impinging on RBCs. This light diffusing in vascularized tissue leads to a broadening of the spectrum of scattered light, from which mean RBC velocity (vel), the blood volume (vol), and blood flow (flow) can be calculated in relative units. In the present study laser Doppler flowmetry was performed at the inferior temporal neuroretinal rim to assess ONH blood flow.

2.3.3. Intraocular pressure

A slit-lamp mounted Goldmann applanation tonometer was used to measure IOP. Before each measurement, one drop of 0.4% benoxinate hydrochloride combined with 0.25% sodium fluorescein was used to achieve local anesthesia of the cornea.

2.3.4. Suction cup method

To increase IOP, the episcleral suction-cup technique first described by Ulrich and co-workers was used (Ulrich and Ulrich, 1985). The eye was topically anesthetized and a rigid standardized 11-mm diameter plastic suction cup was placed on the