L-NAME- and U 46619-induced contractions in isolated porcine ciliary arteries versus vortex veins

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L-NAME- und U 46619-induzierte Kontraktionen in isolierten Ziliararterien und Vortexvenen des Schweines

Hintergrund: Ziel der Arbeit ist es, die durch das Thromboxan A_2 Analogon U 46619 und die durch den Hemmer der Stickstoffmonoxidbildung N^G-nitro-L-Argininmethylester (L-NAME) induzierten Kontraktionen in isolierten Ziliararterien und Vortexvenen des Schweines zu untersuchen.
Material und Methoden: Mit Hilfe eines Myographen, welcher die Messung von isometrischen Kontraktionen ermöglicht, wurden Gefäße 100 mM Kaliumchlorid (KCl) bei steigenden Wandspannungen wiederholt ausgesetzt. Bei ihrer optimalen Wandspannung wurden Gefäße mit funktionstüchtigem Endothel steigenden Konzentrationen von U 46619 (0,1 nM – 1 μM) in Ab- oder Anwesenheit von L-NAME (0,1 mM) ausgesetzt. Die Kontraktionen wurden in mN oder in Prozent der durch 100 mM KCl induzierten Kontraktion angegeben.
Ergebnisse: Die optimale Wandspannung wurde bei 7 mN und 3 mN Vorspannung für Arterien bzw. Venen erreicht. Bei dieser Vorspannung waren die durch KCl induzierten Kontraktionen stärker in Arterien (24,4 ± 3,6 mN; n = 8) als in Venen (1,8 ± 0,2 mN; n = 8). Hingegen waren die durch U 46619 induzierten maximalen Kontraktionen proportional höher (p < 0,001) in Venen (178.3 ± 8.9 %; n = 8) als in Arterien (108,4 ± 2,6 %; n = 5), wurden aber nicht signifikant durch L-NAME beeinflusst. Die Sensitivität auf U 46619 unterschied sich nicht signifikant zwischen Arterien (pD_{50} = 7,7 ± 0,1) und Venen (pD_{50} = 7,9 ± 0,1). Bei Gefäßen im Ruhezustand bewirkte L-NAME Kontraktionen, die höher (p < 0,001) in Venen (43 ± 7,9 %; n = 13) als in Arterien (7,5 ± 1,7 %; n = 10) waren.
Schlussfolgerungen: Im Vergleich zu den durch KCl induzierten Kontraktionen sind sowohl die durch U 46619 als die durch L-NAME induzierten maximalen Kontraktionen proportional höher in Vortexvenen als in Ziliararterien des Schweines.

Schlüsselwörter: Okulärer Blutfluss – Zentralvenenthrombose – Glaukom – Diabetes – Chorioretinopathia centralis serosa

Abstract

Purpose: To investigate contractions evoked by the thromboxane A_2 analog U 46619 and by the inhibitor of nitric oxide formation N^G-nitro-L-arginine methyl ester (L-NAME) in isolated porcine ciliary arteries and vortex veins.
Material and Methods: In a myograph system (for isometric forces measurement), vessels were exposed (at different levels of wall tension) to 100 mM potassium chloride (KCl). At their optimal tension, vessels were exposed (in a cumulative manner) to increasing concentrations of U 46619 (0.1 nM – 1 μM) in the absence or in the presence of L-NAME (0.1 mM). Contractions were expressed in mN or in percent of a 100 mM KCl-induced contraction.
Results: Optimal tension was higher in arteries (7 mN) than in veins (3 mN). Maximal contractions induced by KCl were stronger in arteries (24.4 ± 3.6 mN; n = 8) than in veins (1.8 ± 0.2 mN; n = 8). In contrast, maximal contractions evoked by U 46619 were proportionally higher (p < 0.001) in veins (178.3 ± 8.9 %; n = 8) than in arteries (108.4 ± 2.6 %; n = 5) and were not significantly affected by L-NAME. Sensitivity to U 46619 was not significantly different between arteries (pD_{50} = 7.7 ± 0.1) and veins (pD_{50} = 7.9 ± 0.1). In quiescent vessels, L-NAME evoked contractions that were higher (p < 0.001) in veins (43 ± 7.9 %; n = 13) than in arteries (7.5 ± 1.7 %; n = 10).
Conclusions: When compared with KCl-induced contractions, contractions evoked by U 46619 or L-NAME are proportionally higher in porcine vortex veins than in ciliary arteries.

Key words: Ocular blood flow – central venous thrombosis – glaucoma – diabetes – central serous retinopathy

Changes in the diameter of extraocular arteries or veins regulate intraocular blood flow [1]. Although the vasoactive responses of extraocular arteries have already been studied [2,3,4,5], little is known about the responses of extraocular veins [6,7]. The present study investigates the contractile response to the thromboxane analog U 46619 and the inhibitor of nitric oxide formation N^G-nitro-L-arginine methyl ester (L-NAME) in isolated porcine ciliary arteries and vortex veins.

Klin Monatsbl Augenheilkd 2001; 218: 366 – 369

ISSN 0023-2165

Material and Methods

Vessels' Preparation. Isometric contractions of isolated vessels were studied with a myograph system (Multi Myograph System 610 M and 410 A, J.P. Trading, Aarhus, Denmark). In adherence with the principles of laboratory animal care (NIH publication N° 85-23, revised 1985), porcine eyes were obtained from a slaughterhouse just after death of the animals. Under a microscope (Wild M3C, Heerbrugg, Switzerland) ciliary arteries (ø 200 – 400 µm) and vortex veins (ø 300 – 500 µm) were dissected free and cut into segments (~2 mM). Two tungsten wires (40 µm) were then passed through the lumen and attached to the myograph's force transducer for isometric force measurements [2,3,4,5].

Optimal Passive Tension. Mounted vessels were immersed in the myograph's organ chambers filled with Krebs-Ringer bicarbonate solution (37 °C; 95% O2; 5% CO2) and repeatedly exposed to 100 mM of potassium chloride (KCl) at different levels of wall tension (0.5 – 2 mN steps). The optimal passive tension was defined as the level of vascular wall tension for which contractions to 100 mM KCl became maximal [2,3,4,5].

Endothelial Function. Endothelial function integrity of the vessels was assessed by adding bradykinin (0.3 – 1 µM) on top of a contraction evoked by U 46619 (0.03 – 0.3 µM). It was assumed that the endothelial function was preserved if bradykinin evoked more than 80% relaxation [2,3,4,5]. All experiments have been conducted in vessels with a functional endothelium.

Experimental Protocols. To assess the contractile properties of the thromboxane A_2 analog U 46619, quiescent ciliary arteries and vortex veins with a functional endothelium (set to their optimal passive tension) were exposed, in a cumulative manner, to increasing concentrations of U 46619 (0.1 nM – 1 µM). Experiments were conducted in the absence or in the presence (30 minutes incubation) of either the nitric oxide synthase inhibitor L-NAME (0.1 mM) or the cyclooxygenase inhibitor indomethacin (10 µM).

In another set of experiments, vessels precontracted with U 46619 (0.03 – 0.3 µM) were relaxed by exposing them to increasing concentrations of the nitric oxide donor sodium nitroprusside (SNP; 0.1 nM – 1 mM).

Drugs and Statistical Analysis. U 46619 (9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$), bradykinin, L-NAME, sodium nitroprusside, and indomethacin were purchased from Sigma (Buchs, Switzerland). Indomethacin was dissolved in ethanol. Other drugs were dissolved in distilled water. Concentrations were expressed as final molar concentrations in the organ chambers. Results have been given as mean ± standard error of the mean (mean ± SEM) with n representing the number of eyes studied (one eye per animal). Contractions have been either expressed in mN or in percent of a maximal contraction evoked by 100 mM KCl, relaxations in percent of a precontraction evoked by U 46619 (0.03 – 0.3 µM). The concentrations (expressed as negative log M concentration) of U 46619 evoking 50% contraction (pD_{50}) were calculated. For statistical comparison unpaired Student's t test or an analysis of variance (ANOVA) followed by Scheffe's test were used and a two-tailed P value smaller than 0.05 was considered to be significant.

Results

Optimal Passive Tension. Optimal passive tension was reached at 7 mN in ciliary arteries and 3 mN in vortex veins, respectively (Abb. **1**). At this level of passive tension the maximal contraction evoked by 100 mM KCl was stronger in arteries (24.4 ± 3.6 mN; n = 8) than in veins (1.8 ± 0.2 mN; n = 8).

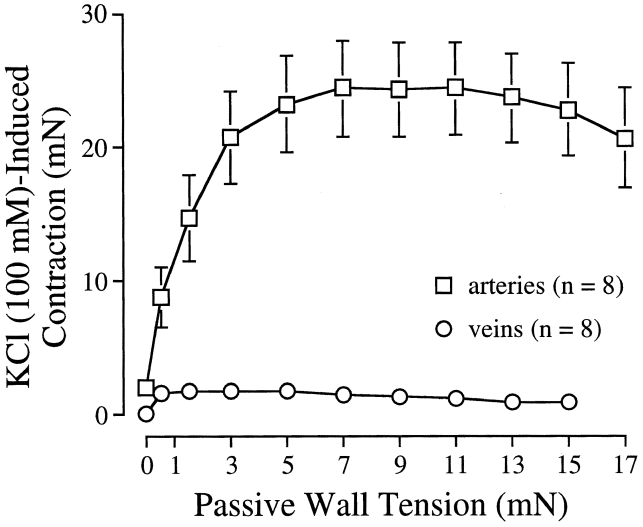

Abb. 1 Effect of increasing passive wall tension (in 0.5 – 2 mN steps) on 100 mM KCl-induced contractions in isolated porcine vortex veins and ciliary arteries. Optimal passive tension was reached at 7 mN and 3 mN for arteries and veins, respectively.

U 46619-Induced Contractions. In quiescent vessels with a functional endothelium, U 46619 (0,1 nM – 1 µM) evoked, in a concentration-dependent manner, marked contractions in veins and in arteries (Abb. **2**). When these contractions were

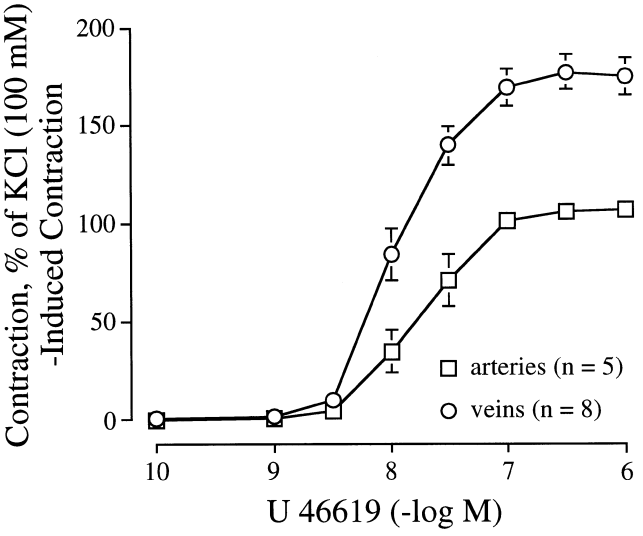

Abb. 2 Effect of increasing concentrations of U 46619 on isolated vortex veins and porcine ciliary arteries. When expressed as a percent of a 100 mM KCl-induced contraction, the maximal contraction induced by U 46619 was proportionally significantly ($p < 0.001$) higher in veins than in arteries.

expressed as a percent of a 100 mM KCl-induced maximal contraction, they appeared to be proportionally ($p < 0.001$) higher in veins (178.3 ± 8.9%; n = 8) than in arteries (108.4 ± 2.6%; n = 5). The sensitivity to U 46619 was not significantly different between arteries ($pD_{50} = 7.7 \pm 0.1$) and veins ($pD_{50} = 7.9 \pm 0.1$). Neither the incubation with L-NAME (0.1 mM) nor with indomethacin (10 μM) had a significant influence on contractions evoked by U 46619 (data not shown).

L-NAME-Induced Contractions. In quiescent vessels the inhibitor of nitric oxide formation, L-NAME (0.1 mM), induced contractions that were ($p < 0.001$) higher in veins (43 ± 7.9%; n = 13) than in arteries (7.5 ± 1.7%; n = 10) (Abb. **3**).

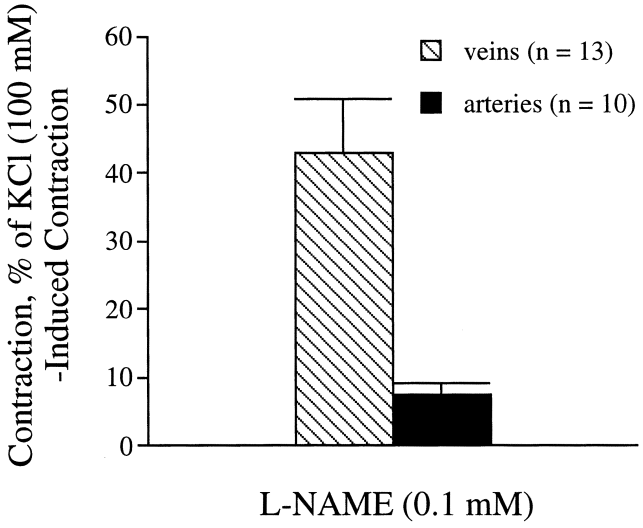

Abb. 3 Effect of single doses of L-NAME (0.1 mM) on isolated porcine vortex veins and ciliary arteries. Maximal contractions observed were significantly higher ($p < 0.001$) in veins than in arteries.

SNP-Induced Relaxations. In vessels precontracted with U 46619 (0.03 – 0.3 μM), SNP (0.1 nM – 1 mM) induced complete relaxations that were not significantly different between arteries and veins (data not shown).

Discussion

This study indicates that in comparison to contractions induced by KCl, the contractions evoked by the thromboxane A_2 agonist U 46619 as well as those induced by the inhibitor of nitric oxide formation L-NAME are more pronounced in porcine vortex veins than in ciliary arteries.

The present study is the first report made in the literature that, within the ophthalmic circulation, some heterogeneity exists in the contractile response between ciliary arteries and vortex veins. An heterogeneity that needs to be further investigated but could reflect a difference in the receptor density or a difference in the mechanism of action of U 46619 between arteries and veins. If such an heterogeneity in contractile response has never been reported in the ophthalmic circulation, differences in the response to different agonists between veins and arteries have been reported in vitro in other vascular beds (human and animal) and in large as well as in small vessels [8,9,10,11,12,13,14].

It has to be noted that contractions evoked by U 46619 were neither affected by the inhibitor of nitric oxide formation, L-NAME, nor by the cyclooxygenase inhibitor indomethacin suggesting that the effect observed was not likely to be due to a production of nitric oxide or prostaglandins.

Contractions evoked by U 46619 were higher than those induced by KCl. This could reflect a difference in the mechanism of action of these two drugs. Indeed, it is usually admitted that KCl contracts vascular smooth muscle cells through a membrane potential depolarization. A depolarization that activates L-type calcium channels (voltage gated) leading to an influx of extracellular calcium [15]. In contrast, contractions evoked by U 46619 are receptor mediated. It has been reported that these contractions do not only result from an influx of extracellular calcium via L-type (voltage gated) calcium channels, but also via non-L-type calcium channels, as well as on mechanisms independent of intracellular calcium elevation [16].

The present study is also the first report made that in the ophthalmic circulation the basal release of nitric oxide is likely to be higher in vortex veins than in ciliary arteries. The exposure of quiescent vessels to the inhibitor of nitric oxide synthase L-NAME evoked contractions that were proportionally more pronounced in veins than in arteries. It is possible that this effect results from a more pronounced basal release of nitric oxide in veins than in arteries. Indeed, a difference of sensitivity to nitric oxide between the two types of vessels could not be observed, as the sensitivity to the vasodilator nitric oxide donor SNP was similar in veins and arteries.

In conclusion, the results presented in this preliminary in vitro study clearly indicate that the maximal contraction induced by U 46619, expressed as a percent of a 100 mM KCl-induced contraction, is proportionally higher in isolated porcine vortex veins than in ciliary arteries. Furthermore the basal nitric oxide release appears to be more sustained in veins than in arteries. These findings might be of potential relevance for the pharmacology and physiology of the ocular blood flow regulation.

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