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Cerebral Arteriogenesis is Enhanced by Pharmacological as Well as Fluid-Shear-Stress Activation of the Trpv4 Calcium Channel

W. Schierling [a,b,c], K. Troidl [a], H. Apfelbeck [a,c], C. Troidl [a], P.M. Kasprzak [b], W. Schaper [a], T. Schmitz-Rixen [c,*]

[a] *Max-Planck-Institute for Heart and Lung Research, Bad Nauheim, Germany*
[b] *Department of Surgery (Vascular and Endovascular Surgery), University Medical Center Regensburg, Regensburg, Germany*
[c] *Department of Vascular and Endovascular Surgery, Johann Wolfgang Goethe-University, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany*

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Abstract *Objectives:* This study aimed to determine the importance of the shear-stress-sensitive calcium channels Trpc1, Trpm7, Trpp2, Trpv2 (transient receptor potential cation channel, subfamily V, member 2) and Trpv4 for cerebral arteriogenesis. The expression profiles were analysed, comparing the stimulation of collateral growth by target-specific drugs to that achieved by maximum increased fluid shear stress (FSS).
Design: A prospective, controlled study wherein rats were subjected to bilateral carotid artery ligature (BCL), or BCL + arteriovenous fistula, or BCL + drug application.
Methods: Messenger RNA (mRNA) abundance and protein expression were determined in FSS-stimulated cerebral collaterals by quantitative real-time polymerase chain reaction (qRT-PCR) and immunohistochemistry. Drugs were applied via osmotic mini pumps and arteriogenesis was evaluated by post-mortem angiograms and Ki67 immunostaining.
Results: Trpv4 was the only mechanosensitive Trp channel showing significantly increased mRNA abundance and protein expression after FSS stimulation. Activation of Trpv4 by 4α-phorbol-12,13-didecanoate caused significantly enhanced collateral growth (length: 4.43 ± 0.20 mm and diameter: 282.6 ± 8.1 μm) compared with control (length: 3.80 ± 0.06 mm and diameter: 237.3 ± 5.3 μm). Drug application stimulated arteriogenesis to almost the same extent as did maximum FSS stimulation (length: 4.61 ± 0.07 mm and diameter: 327.4 ± 12.6 μm).
Conclusions: Trpv4 showed significantly increased expression in FSS-stimulated cerebral collaterals. Pharmacological Trpv4 activation enhanced cerebral arteriogenesis, pinpointing Trpv4 as a possible candidate for the development of new therapeutic concepts.
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* Corresponding author. Tel.: +49 0 69 6301 5349; fax: +49 0 69 6301 5336.
E-mail address: schmitz-rixen@em.uni-frankfurt.de (T. Schmitz-Rixen).

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Introduction

In occlusive cerebrovascular disease, high-grade internal carotid artery stenosis or even complete occlusion can be compensated for by the circle of Willis.[1–3] Adaptation of the collateral circulation may be achieved by arteriogenesis. This term applies to the remodelling of pre-existing arterio-arteriolar anastomoses or arteries outside the ischaemic region, which can replace the capacity of a larger artery.[4–6]As haemodynamic compromise due to insufficient collateral flow is associated with an increased risk of stroke or progressive brain ischaemia,[1–3] new therapeutic options to enhance cerebral arteriogenesis are of considerable importance.

Busch et al. (2003) demonstrated that arteriogenesis can be induced in the adult rat brain by establishing the three-vessel occlusion (3-VO) model (occlusion of one carotid artery and both vertebral arteries).[7] One stimulating factor of cerebral arteriogenesis is the granulocyte-macrophage colony-stimulating factor (GM-CSF). In the 3-VO model, subcutaneous application of GM-CSF led to significant enlargement of the ipsilateral posterior cerebral artery (PCA), a functional improvement of brain haemodynamic parameters and a significant reduction of experimentally induced stroke volume.[8,9] Complete recovery of the cerebrovascular reserve capacity has also been demonstrated after long-term GM-CSF treatment in the bilateral carotid artery occlusion (BCO) model.[10] The proarteriogenic effect of GM-CSF has been explained by the prolongation of the life cycle of monocytes/macrophages, which are important mediators of arteriogenesis.[4,8–11]

Recently, our group has provided evidence that the pivotal trigger of cerebral arteriogenesis is increased intravascular blood flow/fluid shear stress (FSS) and that the growth of cerebral collaterals correlates with rising intravascular flow rate.[12] By analysing the two PCAs of the circle of Willis, as important collaterals after BCO, we demonstrated after 7 days, in the double-ligature model (simultaneous ligature of both common carotid arteries) that blood flow in the two PCAs increases up to 5.0-fold and the diameter of the two PCAs up to 2.2-fold (Fig. 1).[12] A further increase of blood flow and, thereby, vessel growth was reached by additional creation of an arteriovenous (AV) fistula between the distal stump of the occluded common carotid artery and the nearby jugular vein on the left side (ligature-shunt model). In this model, blood flow increased in the 'shunt'-sided PCA up to 7.5-fold and the diameter of the shunt-sided PCA up to 2.9-fold after 7 days (Fig. 1).[12]

One factor involved in translating FSS into a molecular pathway might be the transient receptor potential (TRP) cation channels. These have been implicated in a broad range of functions such as vasomotion, transducers of mechanical, osmotic and thermal stimuli as well as vascular smooth muscle cell (VSMC) proliferation.[13–15]

Thus, the aim of this study was to determine the importance of the known shear-stress-sensitive calcium channels Trpc1 (subfamily C, member 1), Trpm7 (subfamily M, member 7), Trpp2 (subfamily P, member 2), Trpv2 (subfamily V, member 2) and Trpv4 (subfamily V, member 4)[13–15] for cerebral arteriogenesis. The expression profile and stimulation of collateral growth by target-specific drugs compared with stimulation by maximally increased FSS was, therefore, analysed. Mitochondrial RNA (mRNA) and protein expression were detected in the shear-stress-stimulated cerebral collateral circulation at different time points. Drugs were applied via osmotic mini pumps, and the resulting cerebral collateral growth was compared with the high shear stress ligature-shunt model.

Materials and Methods

Animal models

The present study was performed with the permission of the State of Hessen, according to Section 8 of the German Law for the Protection of Animals and conforming to the *Guide of Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Surgical procedures were carried out under anaesthesia with ketamine (100 mg kg^{-1}) and xylazine (4 mg kg^{-1}) administrated i.p. To prevent pain, buprenorphine (0.03 mg kg^{-1}) was applied.

Shear stress models

Sixty male Sprague–Dawley rats (250–300 g; Charles River Laboratories, Sulzfeld, Germany) were randomly assigned to double-ligature, ligature-shunt and sham groups ($n = 20$ each). Surgery was performed as previously described:[12] bilateral ligature of the common carotid artery (double-ligature) and bilateral ligature of the common carotid artery + AV fistula on the left side (ligature-shunt).[12] Sham-operated animals served as controls (Fig. 1). Cerebral integrity of the animals after this surgical procedure has been confirmed in previous studies by magnetic resonance imaging.[12]

Osmotic mini pump models

Drugs were applied via osmotic mini pumps (Model 2001; Alzet® Osmotic Pumps, Distributor: Charles River Laboratories). The phorbol ester, 4α-phorbol-12,13-didecanoate (4αPDD, 0.1 mg kg^{-1} day^{-1}; Sigma–Aldrich, Taufkirchen, Germany), which is a direct, protein kinase C (PKC)-independent channel modulator of N- and L-type Ca^{2+}-channels and K_{ATP}-channels, was used for Trpv4 activation. This ester has been proven to be a robust, reliable tool to study features of Trpv channels and to probe functional effects of channel activation in *in vivo* systems.[16,17] The classic Trp antagonist, Ruthenium Red (RuthRed, 1.0 mg kg^{-1} day^{-1}; Sigma–Aldrich), which reversibly inhibits inward Trpv4 currents, was used to block the Trpv4-channel.[16,17]

Thirty-three male Sprague–Dawley rats (250–300 g) were outfitted with subcutaneously implanted osmotic mini pumps connected to a catheter. After bilateral ligature of the common carotid arteries, this catheter was positioned in the distal stump of the left common carotid artery with the tip next to the carotid bifurcation, so as not to hinder collateral flow (Fig. 4(A)). Animals were randomly assigned to solvent control, RuthRed (2 mg per pump), and 4αPDD (200 μg per pump) groups ($n = 11$ each).

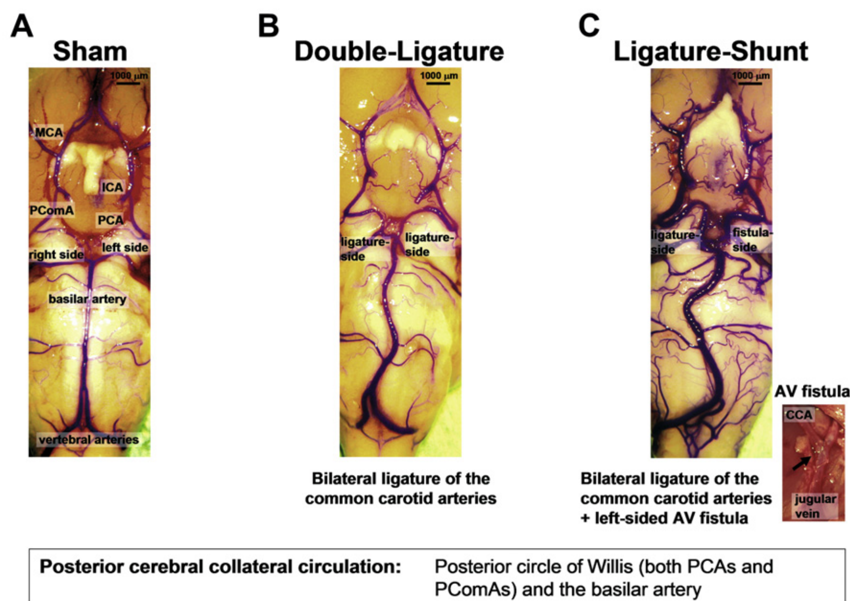

Figure 1 *Collateral growth in the rat brain after 7 days of shear stress-stimulation*. Representative angioarchitecture of the rat brain in *sham*-treated animals (A) and after bilateral ligature of the common carotid arteries (*Double-Ligature*; B) and bilateral ligature of the common carotid arteries + left-sided AV fistula creation (*Ligature-Shunt*; C). AV fistula (C): Arrow points towards the end-to-side anastomosis between the distal stump of the occluded common carotid artery and the nearby jugular vein. PCA (posterior cerebral artery), PComA (posterior communicating artery), ICA (internal carotid artery), MCA (middle cerebral artery), CCA (common carotid artery).

mRNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

After 1, 3 or 7 days of FSS stimulation, rats were euthanised by bleeding after an overdose of ketamine. Total RNA was isolated (RNeasy Micro Kit, Qiagen, Hilden, Germany) from the posterior collateral circulation including both PCAs and posterior communicating arteries and the basilar artery ($n = 5$ each at every point of time). After DNA digestion with DNase-I (Turbo DNAfree, Ambion, Darmstadt, Germany), complementary DNA (cDNA) was synthesised according to Superscript II reverse transcriptase protocol (Invitrogen, Karlsruhe, Germany) using 200 ng total RNA and 200 ng random nonamer oligonucleotides (New England BioLabs® Inc., Frankfurt am Main, Germany).

Gene-specific qRT-PCR primers were selected using FastPCR software (Institute of Biotechnology, University of Helsinki, Finland). qRT-PCR was performed in a 25-μl reaction/96-well format using 1.0 μl cDNA (1:20), 200 nM each primer, and 1× iQ SYBR Green Super Mix (Bio-Rad, Laboratories, München, Germany) in an iCycler RT-PCR system (Bio-Rad Laboratories). Samples were measured in triplicate, with a minimum of two independent experiments. Relative amount of target mRNA normalised to 18S RNA was calculated as previously described.[18]

Immunohistochemistry

For Trpv4 staining (shear stress models), rats were euthanised 7 days after surgery ($n = 5$) and for Ki67 staining (osmotic mini pump models), 3 and 5 days after surgery ($n = 3$ each, at every time point). Immunostaining of both PCAs and the basilar artery was performed on 5-μm cryosections, as previously described.[12]

The following antibodies/agents were used: Trpv4 (1:100, Alomone Labs Ltd., Jerusalem, Israel), CD31 (1:100, Antigenix America Inc., Huntington Station, NY, USA), Ki67/MIB-5 (1:20, Dako, Hamburg, Germany); Biotin-SP-conjugated donkey anti-rabbit immunoglobulin G (IgG) (Dianova, Hamburg, Germany), Cy2-conjugated Streptavidin (Biotrend, Köln, Germany), Cy3-conjugated anti-mouse IgG (Chemicon/Millipore, Schwalbach, Germany), Phalloidin fluorescein isothiocyanate (FITC) labelled (actin staining; Sigma—Aldrich) and Draq5 (nuclei staining; Alexis® Biochemicals, Lörrach, Germany). Sections were viewed with a confocal scanning laser microscope (Leica TCS SP), as previously described.[12]

The quantification of Trpv4 fluorescence intensity was performed with representative images, using ImageJ 1.38p software (http://rsb.info.nih.gov/ij/), as previously described.[19] A full range of grey values, from black to peak white (0-pixel—255-pixel intensity level), was set during measurements. Fluorescence intensity was expressed as arbitrary units per square micrometre (AU μm^{-2}). For determination of the proliferation index in growing basilar arteries, Ki67-positive nuclei of endothelial cells (ECs) and smooth muscle cells (SMCs) were counted and referred to the total number of nuclei of each particular vessel layer.

Cerebral post-mortem angiograms

Diameter and vessel length of both PCAs were analysed to evaluate cerebral arteriogenesis after 7 days of treatment

with RuthRed, 4αPDD and solvent control versus sham ($n = 5$ each), as previously described,[12] and compared with the collateral growth after 7 days of increased shear-stress stimulation in the ligature-shunt model. Briefly, after maximum adenosine-induced vasodilatation, the cerebrovascular circulation of euthanised rats was perfused with prewarmed (37 °C), gelatine/barium-based contrast medium via the right carotid artery at a physiological pressure of 80 mmHg. Pictures were taken using a digital camera mounted on a Carl Zeiss microscope (OPMI® 1 FR). Vessel length was measured between stem and re-entry, and external diameters at three different levels using ImageJ 1.38p software (http://rsb.info.nih.gov/ij/).

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). The D'Agostino and Pearson omnibus normality test was used to test normal distribution, group differences were analysed for statistical significance using one-way analysis of variance (ANOVA) and *post hoc* comparisons were performed using a Bonferroni correction (Prism, GraphPad Software, Inc.). A p value <0.05 was considered statistically significant.

Results

Trpv4 is the only one of the known shear-stress-sensitive Trp-channels with increased expression on the transcriptional and translational levels

mRNA was isolated from the posterior collateral circulation of the double-ligature and ligature-shunt groups (Fig. 1) and qRT-PCR was performed for the known shear-stress-sensitive Trp channels Trpc1, Trpm7, Trpp2, Trpv2 and Trpv4 at 1,3 and 7 days after surgery ($n = 5$ each, at every time point; Fig. 2).

Evaluation of Trpc1, Trpm7, Trpp2 and Trpv2 failed to demonstrate elevated mRNA levels after shear stress stimulation in the double-ligature as well as in the ligature-shunt group (Fig. 2(A)—(D)). By contrast, elevated expression of Trpv4 mRNA versus sham-operated controls was found in the double-ligature group on day 1 (2.3 ± 0.2-fold), which increased to 3.1 ± 0.3-fold and 3.0 ± 0.3-fold on days 3 and 7, respectively (Fig. 2(E); $p < 0.05$). Even higher mRNA expression was seen after further increased shear-stress stimulation in the ligature-shunt group up to 3.4 ± 0.2-fold on day 1 and, maximally, up to 4.8 ± 0.5-fold on day 3 (Fig. 2(E); $p < 0.05$ vs. control and double-ligature groups).

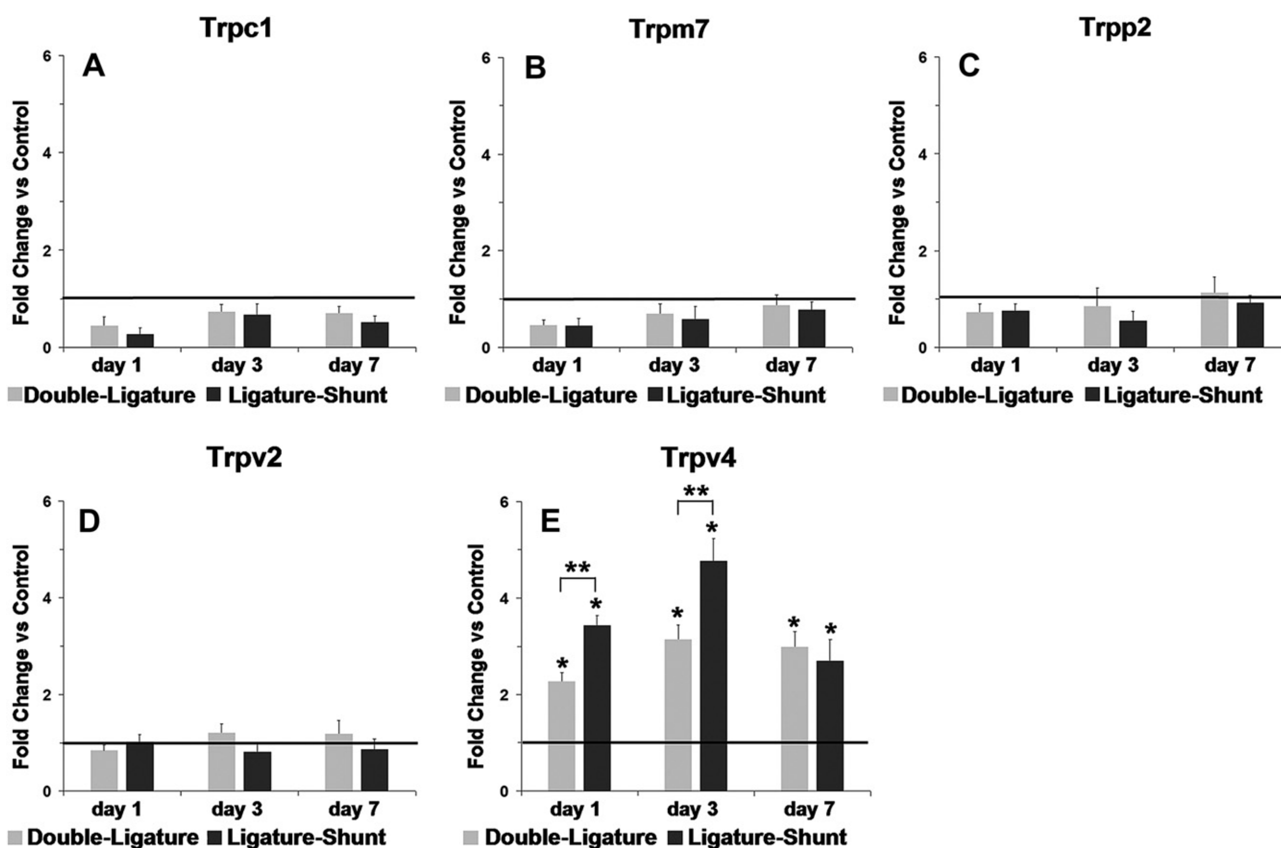

Figure 2 *Expression profile of known shear-stress-sensitive Trp-channels in the FSS-stimulated cerebral collateral circulation.* mRNA-abundance of Trpc1 (A), Trpm7 (B), Trpp2 (C), Trpv2 (D), and Trpv4 (E) in the posterior collateral circulation after 1, 3, and 7 days of FSS stimulation in the *Double-Ligature* and *Ligature-Shunt* group using qRT-PCR ($n = 5$ each at every time point). Relative numbers of transcripts were measured in fold-changes vs *sham*-treated controls (= 1, black line). Values are expressed as mean ± SEM; *$p < 0.05$ vs *sham*; **$p < 0.05$.

Immunostaining of the PCAs was performed in the double-ligature and ligature-shunt groups at day 7 to further localise Trpv4 protein expression in the vessel wall ($n = 5$ each; Fig. 3).

Basal Trpv4 protein expression was detected in the vessel wall of sham-operated controls (Fig. 3(A) and (B)). Distinctly increased Trpv4 protein expression, restricted to the endothelium, was determined in ligature-treated PCAs (8.4 ± 1.5 AU μm^{-2} ligature vs. 2.0 ± 0.9 AU μm^{-2} sham; $p < 0.05$; Fig. 3(A)–(B)). Maximal fluorescence-intensity for Trpv4-protein expression in the endothelium was found after maximal shear-stress stimulation at the 'shunt'-sided PCA of the ligature-shunt group (19.4 ± 4.7 AU μm^{-2}; $p < 0.05$ vs. sham and ligature groups; Fig. 3(A) and (B)).

Cerebral arteriogenesis is considerably enhanced by pharmacological activation of the mechanosensitive calcium channel Trpv4

Trpv4 activity was modulated *in vivo* after bilateral carotid ligature by additional application of 4αPDD or RuthRed via osmotic mini pumps for a period of 7 days (bilateral carotid artery ligature (BCL) + drug application; Fig. 4(A)). 4αPDD is a non-PKC-activating phorbol ester and a subtype-selective Trpv4 agonist of Trpv4. The classic Trp inhibitor, Ruthenium Red, reversibly inhibits inward but not outward Trpv4 currents and is also an antagonist of other Trpv channels. The resulting impact on cerebral arteriogenesis was compared with the high shear stress, ligature-shunt model (BCL + left-sided AV fistula). To quantify cerebral arteriogenesis, length and diameter of the PCAs were determined by post-mortem angiograms after maximum adenosine-induced vasodilatation ($n = 5$ each; Fig. 4). The normal angioarchitecture of the unstimulated PCAs is shown in sham-operated animals (Fig. 4 (B) and (C)).

Analysis of the length of the PCAs showed a significant increase after 7 days of 4αPDD treatment versus solvent control (4.43 ± 0.20 vs. 3.80 ± 0.06 mm; $p < 0.05$; Fig. 4(B) and (C)). The increase almost reached the same level as that achieved after 'shunt' treatment (4.61 ± 0.07 mm; Fig. 4 (B) and (C)). Treatment with RuthRed significantly decreased vessel length compared with solvent control (3.38 ± 0.09 mm vs. 3.80 ± 0.06 mm; $p < 0.05$; Fig. 4(B) and (C)).

Cerebral arteriogenesis, assessed by vessel diameter, was significantly enhanced by 4αPDD treatment compared with solvent control (282.6 ± 8.1 μm vs. 237.3 ± 5.3 μm; $p < 0.05$; Fig. 4(B) and (C)). However, values as high as those reached after 'shunt' treatment were not achieved (327.4 ± 12.6 μm; $p < 0.05$; Fig. 4(B) and (C)). Treatment

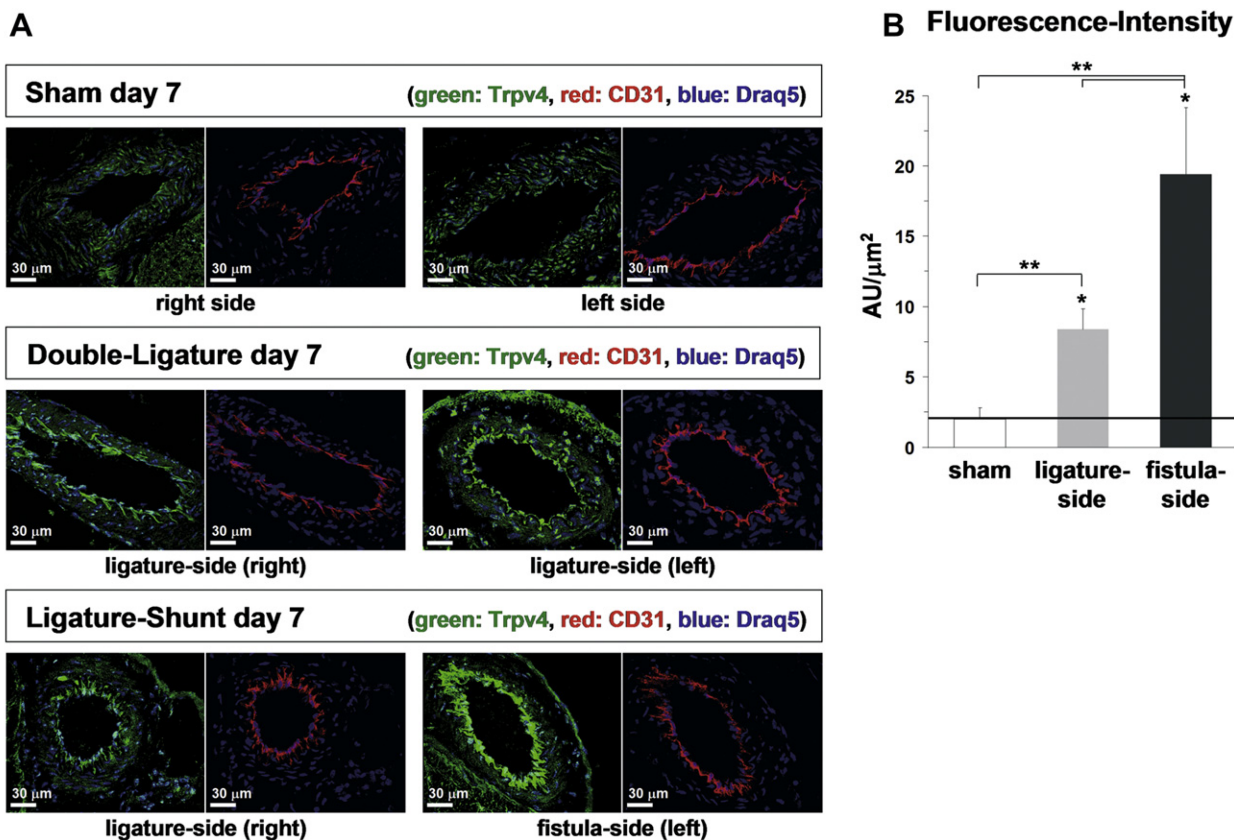

Figure 3 *Shear stress-sensitive response of Trpv4 protein-expression in the posterior cerebral arteries.* (A), Trpv4-immunostaining of the posterior cerebral arteries after 7 days of FSS stimulation in the *Double-Ligature* and *Ligature-Shunt* group compared to *sham*-treated controls. Representative images: Trpv4: green; CD31 (endothelial cells): red; Draq5 (nuclei): blue. (B), Quantification of fluorescence-intensity, given in arbitrary units (AU)/μm^2, by ImageJ 1.38p software. Values are expressed as mean ± SEM; $n = 5$ each; *$p < 0.05$ vs *sham*; **$p < 0.05$.

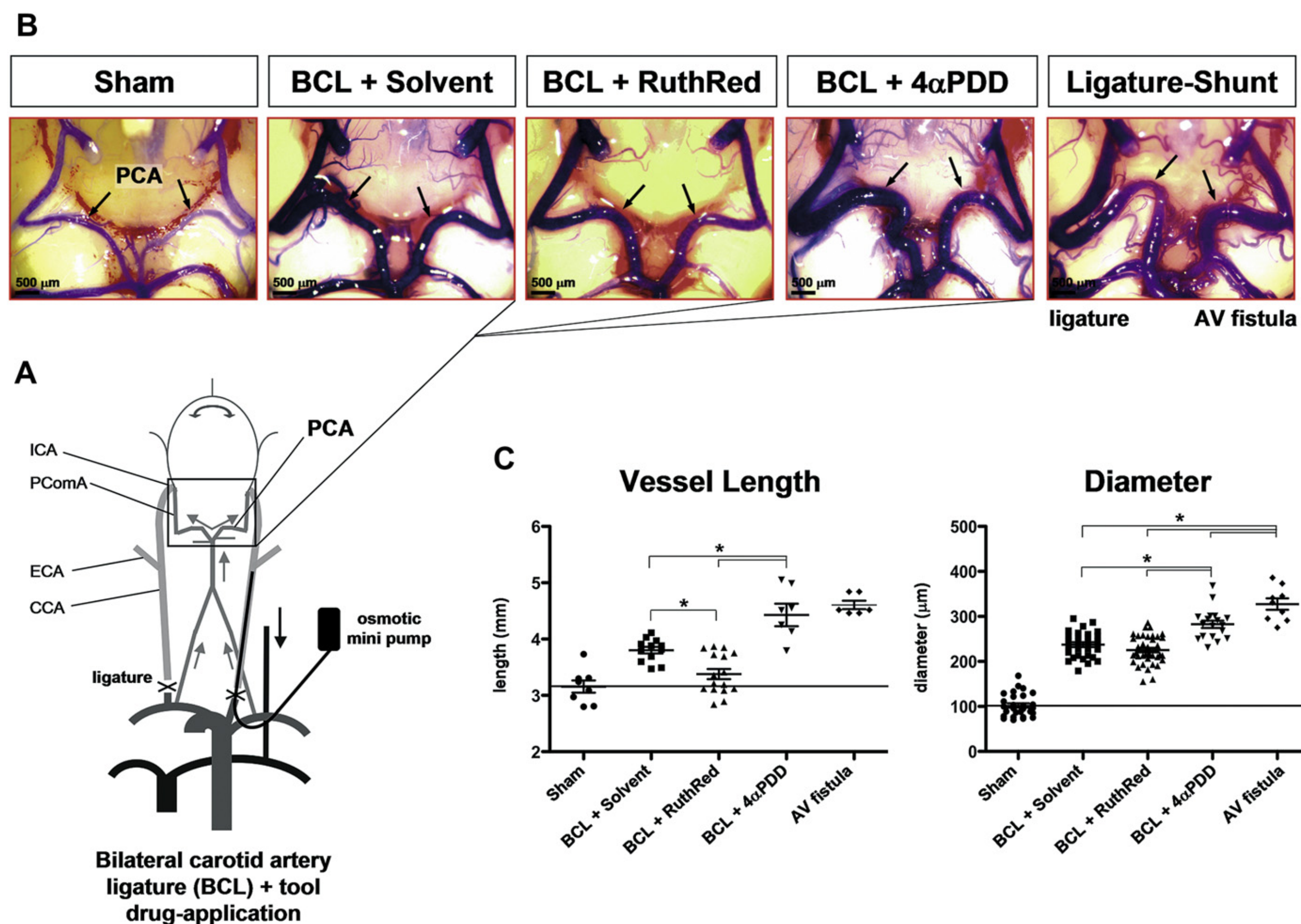

Figure 4 *Influence of 4αPDD-/RuthRed-treatment on cerebral arteriogenesis after 7 days*. (A), Schematic diagram of the osmotic mini pump model. ICA (internal carotid artery), ECA (external carotid artery), CCA (common carotid artery), PComA (posterior communicating artery), PCA (posterior cerebral artery). (B), Representative images of the posterior cerebral arteries (PCAs) after bilateral carotid ligature (BCL) + 7 days of *4αPDD*- or *RuthRed*-treatment vs *Solvent Control* and compared to *sham*- and *Ligature-Shunt*-operated animals. Arrows point towards the posterior cerebral arteries. (C), Quantitative analysis of post-mortem angiograms on vessel length and diameter of the posterior cerebral arteries after BCL + 7 days of *4αPDD*- or *RuthRed*-treatment vs *Solvent Control*, compared to *sham*- and *Ligature-Shunt*-operated animals. Values are expressed as mean ± SEM; $^*p < 0.05$; $n = 5$ each.

with RuthRed showed only a trend to reduce vessel diameter in comparison to solvent control (Fig. 4(B) and (C)).

Ki67 immunostaining of the A. basilaris was performed after 3 and 5 days of 4αPDD- or RuthRed treatment to monitor the proliferation activity of ECs and SMCs *in vivo* ($n = 3$ each at every point of time; Fig. 5). The left panel in Fig. 5(A) and (B) demonstrates the normal proliferation of vascular cells in the unstimulated basilar artery of sham-operated animals (Fig. 5(A)–(C)).

Proliferation activity of the ECs (from day 3) and SMCs (from day 5) was significantly increased after Trpv4 activation by 4αPDD compared with solvent control. Application of RuthRed significantly decreased proliferation activity of vascular cells (Fig. 5(A)–(C): ECs on day 3: 70.5 ± 1.5% *4αPDD* vs. 31.3 ± 5.3% solvent control vs. 8.0 ± 4.5% RuthRed; ECs on day 5: 38.4 ± 5.2% *4αPDD* vs. 16.9 ± 2.5% solvent control vs. 9.5 ± 2.7% RuthRed; SMCs on day 5: 13.0 ± 3.6% *4αPDD* vs. 5.5 ± 2.1% solvent control vs. 5.8 ± 2.5% RuthRed; $p < 0.05$).

Discussion

Insufficient cerebral collateral blood flow increases the risk of stroke or progressive brain ischaemia.[1–3] Therefore, it is important to establish new therapeutic options to enhance collateral growth in the brain. In previous studies, we have demonstrated that FSS is the pivotal trigger of cerebral arteriogenesis.[12] Consequently, tracing the molecular pathway of increased FSS could open an avenue to pharmacological enhancement of cerebral arteriogenesis. Relevant candidates are the TRP cation channels, which are expressed in almost every tissue, including the heart and vasculature.[20] Most of the 28 mammalian Trp channels are permeable to Ca^{2+}, act as multifunctional cellular sensors and are involved in fundamental cell functions such as contraction, proliferation and cell death.[20] Hence, the subject of this study was to investigate the known shear-stress-sensitive calcium channels Trpc1, Trpm7, Trpp2, Trpv2 and Trpv4,[13–15] and evaluate their importance for cerebral arteriogenesis.

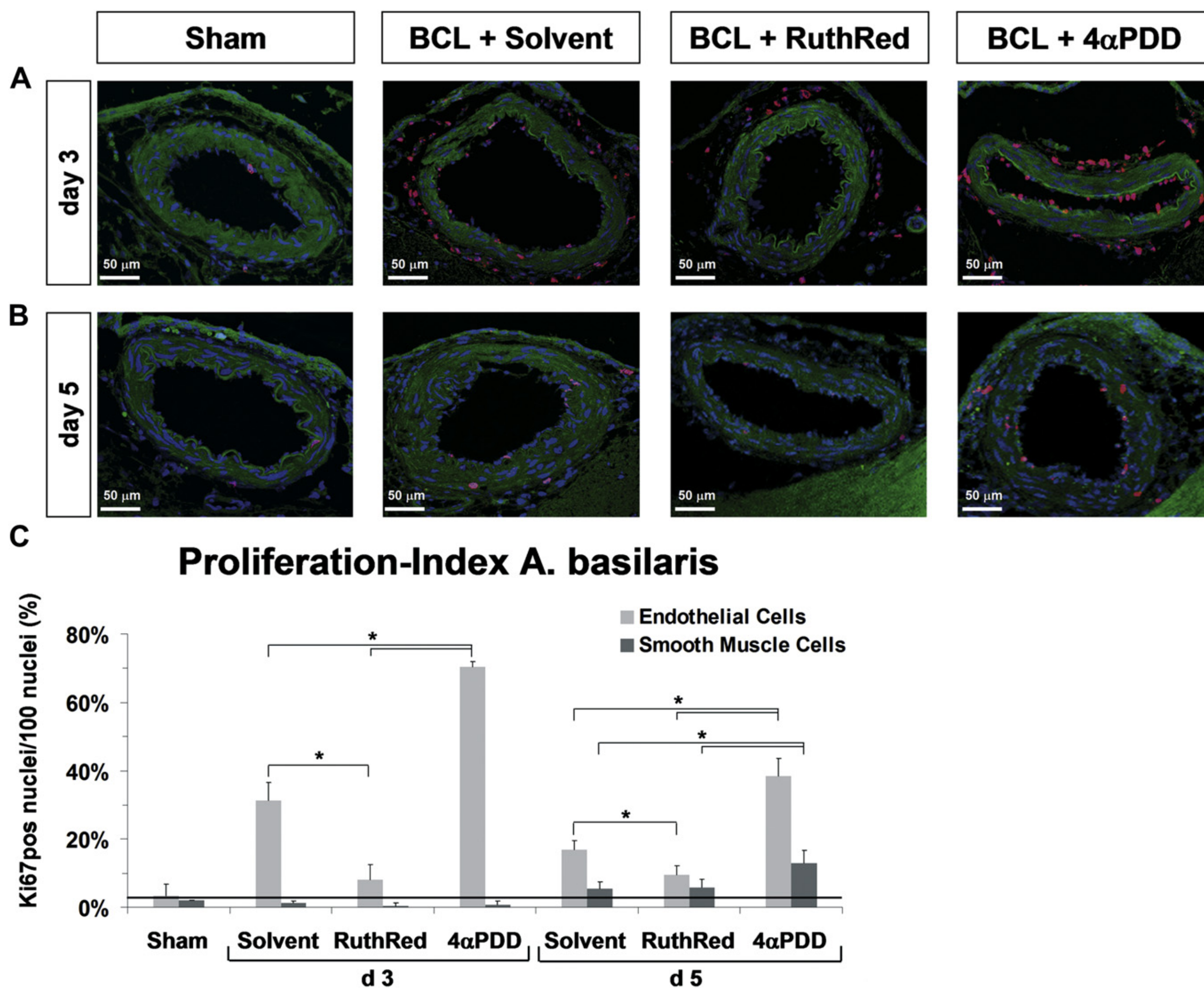

Figure 5 *Proliferation activity in cerebral collaterals after 4αPDD-/RuthRed-treatment*. Ki67-immunostaining of the basilar artery after bilateral carotid ligature (BCL) + 3 days (A) and 5 days (B) of *4αPDD-* and *RuthRed*-treatment vs *Solvent Control*, compared to *sham*-operated animals. Representative images: Ki76: red, Phalloidin-FITC (actin): green, Draq5 (nuclei): blue. (C), Proliferation activity of endothelial and smooth muscle cells in the basilar artery after BCL + 3 days and 5 days of *4αPDD-* and *RuthRed*-treatment vs *Solvent Control*, compared to *sham*-operated animals (black line). Values are expressed as mean ± SEM; $*p < 0.05$; $n = 3$ each, at both time points.

Smooth muscle and endothelium are remarkably diverse, with major structural and functional heterogeneity apparent between organs and along vascular segments.[20] So far, little is known about how this diversity is maintained and how site-specific responses are determined.[20] In the present study, Trpv4 was the only one of the known mechanosensitive Trp channels with increased mRNA and protein expression after shear-stress stimulation.

In accordance with other studies,[15] we found Trpv4 highly expressed in endothelial cells. Moreover, the expression of the Trpv4 protein in the endothelium was highly shear stress-sensitive. Therefore, insufficient adaptation to occlusion or stenosis may lie in premature normalisation of FSS, due to the fact that increasing collateral diameter reduces FSS by the third power of the radius.[21] This effect, which could not be fully compensated for despite AV fistula creation, might account for decreasing Trpv4 mRNA levels at 7 days after surgery, as opposed to higher levels after 3 days. A relevant new finding of the present study is that cerebral arteriogenesis can be significantly enhanced, not only by FSS, but also pharmacologically by application of 4αPDD. Compared with maximum AV-fistula-induced FSS stimulation,[12] the number of proliferating ECs after 4αPDD treatment was even higher. The increase of vessel length and diameter reached approximately 86–96% of the maximum values achieved after shunt-treatment. An explanation for the submaximal 4αPDD stimulation of arteriogenesis, compared with FSS stimulation, is that mechanical FSS may stimulate additional pathways such as NO signalling,[22,23] which remain unstimulated by 4αPDD. The incomplete growth inhibition by Ruthenium Red might be due to the fact that it is not a specific Trpv4 antagonist, but also inhibits mitochondrial Ca^{2+}-uniporter and other Trpv channels.[13–15]

The importance of Trpv4 and the possibility of pharmacological activation has been established in previous studies on

peripheral arteriogenesis.[19,24] This is relevant because there are major differences in physiology, anatomy and morphology between cerebral and peripheral collaterals.[20] The differences are reflected in low-resistance vessels in the brain versus high-resistance vessels in the hindlimb and the number of visible collaterals. Experiments have shown that two of the most highly expressed genes in peripheral arteriogenesis, namely the cardiac ankyrin repeat protein and the actin-binding Rho activating protein,[22] were not upregulated in cerebral arteriogenesis after shear-stress stimulation (unpublished data). This highlights the calcium channel Trpv4 as a drug target of special interest in developing new therapeutic concepts.

Downstream signalling, in particular Ca^{2+}-dependent transcriptional activity of TRPV4 activation, has previously been studied. Three major calcium-dependent transcriptional effectors (nuclear factor of activated T-cells (NFAT), myocyte enhancer factor2 (MEF2) and CSEN) as well as CaN, activator protein-1 (AP-1) and CSEN are modulated during 4αPDD-triggered or AV-shunt-induced peripheral arteriogenesis in pigs.[24]

One limitation of the present study is that functional investigations on the cerebral haemodynamic reserve capacity were not determined. Another limitation is that 4αPDD cannot be used for human patients. However, this is the first demonstration of the importance of the shear-stress-sensitive calcium channel Trpv4 for cerebral arteriogenesis.

Conclusions

The endothelially expressed and highly shear-stress-sensitive Trpv4 calcium channel has proven to be an important factor in the remodelling of collaterals during cerebral arteriogenesis. Pharmacological activation of Trpv4 can enhance cerebral arteriogenesis. Trpv4, as an endothelial membrane-bound protein, may be an interesting new target for the development of new therapeutic concepts in treating cerebrovascular disease.

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Conflict of Interest

The authors declare no conflicts of interest.

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