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

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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 $\pm$ 0.20 mm and diameter: 282.6 $\pm$ 8.1 μm) compared with control (length: 3.80 $\pm$ 0.06 mm and diameter: 237.3 $\pm$ 5.3 μm). Drug application stimulated arteriogenesis to almost the same extent as did maximum FSS stimulation (length: 4.61 $\pm$ 0.07 mm and diameter: 327.4 $\pm$ 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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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).