Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
Molecular Pharmacology Fast Forward. Published on February 6, 2008 as doi:10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

MOL#43323

Tissue specific regulation of microvascular diameter: opposite functional roles of neuronal and smooth muscle located vanilloid receptor-1 (TRPV1)

Tamás Kark, Zsolt Bagi, Erzsébet Lizanecz, Enikő T. Pásztor, Nóra Erdei, Ágnes Czikora, Zoltán Papp, István Édes, Róbert Pórszász[1], Attila Tóth[1]

Department of Pharmacology and Pharmacotherapy, Institute of Pharmacology (T.K., R.P.) and Division of Clinical Physiology, Institute of Cardiology (Z.B., E.L., E.T.P., N.E., Á.C., Z.P., I.É., A.T.), Medical and Health Science Center, University of Debrecen, Debrecen, Hungary.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Copyright 2008 by the American Society for Pharmacology and Experimental Therapeutics.

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

MOL#43323

Running title: TRPV1 expression in vascular smooth muscle cells

Corresponding author:

Attila Tóth, PhD

Division of Clinical Physiology, Institute of Cardiology, Medical and Health Science Center, University of Debrecen

22 Moricz Zs krt, 4032, Debrecen, Hungary

Tel.: (36) 52-414-928

Fax: (36) 52-323-978

E-mail: atitoth@dote.hu

Number of text pages: 30

Number of tables: 0

Number of figures: 7

Number of references: 39

Number of words:

In the abstract: 248

In the introduction: 296

In the discussion: 1182

Abbreviations

TRPV1 or VR1: vanilloid (capsaicin) receptor 1

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

The vanilloid receptor 1 (TRPV1) is a Ca^{2+}-permeable nonspecific cation channel, activated by various painful stimuli including ischemia. We hypothesized that TRPV1 is expressed in the arterioles and involved in the regulation of microvascular tone. We found that TRPV1 stimulation by capsaicin (intra-arterial administration) of the isolated, perfused right hindlimb of the rat increased vascular resistance (by 98±21 mmHg at 10 μg), in association with decreased skeletal muscle perfusion and elevation of skin perfusion (detected by dual channel laser Doppler flowmetry). Denervation of the hindlimb did not affect capsaicin-evoked changes in vascular resistance and tissue perfusion in the hindlimb, but reduced elevation of perfusion in the skin. In isolated, pressurized skeletal (m. gracilis) muscle arterioles (diameter: 147±35 μm), capsaicin had biphasic effects: at lower concentrations of capsaicin (up to 10 nmol/L) evoked dilations (max: 32±13%), while higher concentrations (0.1-1 μmol/L) elicited substantial constrictions (max: 66±7%). Endothelium removal or inhibition of nitric oxide synthase abolished capsaicin-induced dilations, but did not affect arteriolar constriction. Expression of TRPV1 was detected by RT-PCR in the aorta and in cultured rat aortic vascular smooth muscle cells (A7r5). Immunohistochemistry revealed expression primarily in the smooth muscle layers of the gracilis arteriole. These data demonstrate the functional expression of TRPV1 in vascular smooth muscle cells mediating vasoconstriction of the resistance arteries. Due to the dual effects of TRPV1 stimulation on the arteriolar diameter (dilation in skin, constriction in skeletal muscle), we propose that TRPV1 ligands represent drug candidates for tissue specific modulation of blood distribution.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

The transient receptor potential type V1 channel (vanilloid receptor-1, TRPV1) is a non-selective cation channel, structurally belonging to the transient receptor potential family (TRP) of ion channels. TRPV1 is found in sensory C and A-δ fibers (Caterina *et al.*, 1997) and functions as a ligand-, proton- and heat-activated molecular integrator of nociceptive stimuli, in the periphery (Di Marzo *et al.*, 2002b;Di Marzo *et al.*, 2002a;Ross, 2003;Szallasi and Blumberg, 1999). Activation of TRPV1 leads to central (pain) as well as to local 'sensory-efferent' effects (Szolcsanyi, 1988).

It is well established that the sensory-efferent effects of TRPV1 stimulation include the release of neuropeptides such as calcitonin gene related peptide (CGRP) and substance P (SP) from the sensory nerve terminals. These peptides cause vasodilatation in different vascular beds such as mesenteric, hepatic, basilar, dural and meningeal arterioles (Akerman *et al.*, 2004;Dux *et al.*, 2003;Harris *et al.*, 2002;O'Sullivan *et al.*, 2004;Ralevic *et al.*, 2001;Zygmunt *et al.*, 1999). Interestingly, TRPV1-induced release of SP from sensory neurons has been recently implicated in mediating pressure-induced myogenic constriction (Scotland *et al.*, 2004). Similarly, previous studies have also proposed that in certain circumstances TRPV1 activation may lead to vasoconstriction in mesenteric (Porszasz *et al.*, 2002), coronary (Szolcsanyi *et al.*, 2001), skeletal muscle (Lizanecz *et al.*, 2006) and dural vessels (Dux *et al.*, 2003), although the underlying mechanism remained obscure.

In the present study the possible mechanisms of TRPV1 mediated vascular effects were investigated. It was found that TRPV1 stimulation results in opposite effects in different arterial beds from the same hindlimb of the rat, *in vivo*, namely vasodilation in

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

the skin and vasoconstriction in the skeletal muscle. Moreover, investigation of the possible mechanisms of TRPV1 mediated responses confirmed TRPV1 expression in vascular smooth muscle cells and suggested cell type specific differences in the capsaicin responsiveness.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Materials and Methods

Animals, anaesthesia and general preparation in the *in vivo* experiments

The experiments were performed on male Wistar rats weighing 250-450 g raised on a standard laboratory food and water ad libitum. Anaesthesia was performed with 100 mg/kg i.p. thiobutobarbital (Inactin, Byk). The right common carotid artery and the left internal jugular vein were cannulated with polyethylene tubing for continuous measurement of arterial blood pressure and for administration of drugs, respectively. Respiratory movements were measured by means of a Statham transducer connected to one side of a Y-shaped cannula introduced into the trachea. The body core temperature was maintained around 37 °C with a temperature controlled infrared heating lamp. Recordings were displayed on a polyphysiograph. All procedures used in this study are in agreement with the rules of the Ethics Committee on Animal Research.

Hindlimb autoperfusion and recording of perfusion pressure

Isolated hindlimb autoperfusion and perfusion pressure recording were performed as described previously (Colquhoun *et al.*, 1988). Briefly, after administration of heparin sodium (1000 U/kg i.v.), the right hindlimb was perfused by means of a peristaltic pump (Masterflex, model 7519-20, Cole-Parmer Instruments, U.S.A.) with blood taken from a catheter inserted in the left common carotid artery. The outlet side of the perfusion circuit (Masterflex Silicon tube platinum, 96410-14) was connected to the common iliac artery, which was approached through a midline abdominal incision. Perfusion pressure, measured from a side arm in the perfusion circuit between the pump and the iliac artery, was recorded using a Spectramed P23XL pressure transducer and displayed on a

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

recorder. During the whole surgical procedure the ischaemic period of the leg never lasted longer than 3-5 min. The peristaltic pump was set to produce a constant flow rate (3 ml/min). This arrangement enabled us to record reproducible responses over 2-3 hours without swelling of the paw. Capsaicin (8-Methyl-N-vanillyl-*trans*-6-nonenamide), norepinephrine ((±)-4-(2-Amino-1-hydroxyethyl)-1,2-benzenediol hydrochloride) and oxytocin (α-Hypophamine) were administered into the perfusion cannula near to the iliac artery catheterization site. For administration a Hamilton syringe was used and the injected volumes varied between 10-100 μl. Acute cross-section of femoral, genitofemoral and sciatic nerves were carried out in the right hindlimb to prevent nociceptive reflexes from the perfused leg.

Measurement of skin and striated muscle blood flow

Laser-Doppler recordings of microvascular blood flow changes were made in the middle of the paw covered by thin glabrous skin and from the flexor muscles of the thigh using a dual channel laser Doppler flowmeter (MBF3D, Moor Instruments Ltd., UK). The time constant was set to 1 s. During the experiment the exposed skeletal muscle was kept in moist by a wet chamber placed around the probe. Blood flow changes were recorded continuously throughout the experiment and were expressed as arbitrary units of flux (Escott and Brain, 1993;Porszasz and Szolcsanyi, 1994). The zero level was verified at the end of the experiment. Disturbances caused by direct light were excluded by means of a piece of cotton wool placed onto the right hindlimb. The peak of the changes was used to calculate the effect of drugs.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Chronic denervation of hindlimb

In a group of animals (n= 15) the nervous supply of the right hind leg was denervated 7-20 days before the experiment under sodium pentobarbital (5-Ethyl-5-(1-methylbutyl)-2,4,6-trioxohexahydropyrimidine) anaesthesia (40 mg/kg, i.p.), as described previously (Santha and Jancso, 2003). Briefly, an incision was performed on the abdomen and the right femoral, genitofemoral and sciatic nerves were crossected transperitoneally. Then hemostasis was confirmed and the wound was sutured. During recovery from the anaesthesia the animals were placed under an infrared heating lamp. Animals showing autotomy were not included in the experiments.

Isolation of arterioles and experimental protocols

The isolation of the skeletal (gracilis) muscle arterioles of the rat and diameter measurement of the arterioles were performed as described earlier (Lizanecz *et al.*, 2006). Briefly, after spontaneous tone developed in response to intraluminal pressure of 80 mmHg, arteriolar responses were obtained in maximal response to cumulative doses of the TRPV1 agonist, capsaicin (0.1 nmol/L - 1 μmol/L). Capsaicin-induced responses were also observed after endothelium removal (Koller and Bagi, 2004) or after inhibition of NO synthase with L-NAME (N-Nitro-L-Arginine Methyl Ester) (Koller and Bagi, 2004). In separate experiments intraluminal pressure was changed from 20 to 120 mmHg and changes in diameter were measured before and after capsaicin treatment (1 μmol/L for 20 minutes followed by a 40 minutes regeneration period) (Scotland *et al.*, 2004).

Immunhistochemistry

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

The immunohistochemical experiments were performed as described by Lizanecz et al.[16] with minor modifications. In short, m. gracilis, skin and small mesenteric tissues were dissected from Wistar rats and were embedded in Tissue-Tek O.C.T compound (Electron Microscopy Sciences, Hatfield, PA, USA). Cryostat sections (thickness 10 μm) were placed on adhesive slides and fixed in acetone for 10 min. The slices were blocked with normal goat sera (1.5% in PBS, Sigma, St. Louis, MO, USA) for 20 min and stained with anti-capsaicin receptor antibodies (Chemicon: AB 5370P (rabbit) and AB 5566 (guinea pig); Calbiochem: PC 547 (rabbit); Neuromics: RA 10110 (rabbit) and GP 14100 (guinea pig)) at a 1:500 dilution (for all TRPV1 specific antibodies), with smooth muscle actin antibody (NCL-SMA, Novocastra Laboratories, New Castle, UK, dilution: 1:20) or with a neurofilament specific antibody (Sigma, St. Louis, MO, USA, dilution: 1:100) in the blocking buffer. Then, the slices were incubated with anti rabbit, mouse and guinea pig antibodies conjugated with Texas red or Cy2. The pictures were captured by a (Scion Corp., Frederick, MA, USA) digital camera attached to a Nikon Eclipse 80i fluorescent microscope.

Detection of TRPV1 mRNA

Total RNA was isolated from rat aorta and A7r5 cells (obtained from American Type Culture Collection (LGC Promochem, Wesel, Germany) and maintained in 10% FBS containing DMEM, both Life Technologies Inc., Rockville, MD, USA) with RNeasy RNS isolation kit (Qiagen GmbH, Hilden, Germany) and cDNA was synthesized by a RevertAid H Minus kit (Fermentas UAB, Vilnius, Lithuania), according to the manufacturer's instruction. The RT-PCR was performed by a sense

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

(5'CTACCTGGAACACCAATGTGGG3') and an antisense primer (5'GCTGGGTGGCATGTCTATCTCG3') designed to produce a 596 bp fragment from DNA and a 149 bp fragment from RNA. Glycerol aldehyde phosphate dehydrogenase (GAPDH) was used as control. PCR reaction was performed in a volume of 25 µl consisting of: 1 µl cDNA, 1 µM primer, 200 µM dNTP, 6 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3) and 2,5 U Long PCR Enzyme Mix (Fermentas UAB, Vilnius, Lithuania). The protocol was 94 °C 5 min, followed by 35 cycles of 20 s 94 °C; 20 s 58 °C and 40 s 72 °C.

Materials and solutions

Capsaicin, norepinephrine and oxytocin (α-Hypophamine) Capsaicin (8-Methyl-N-vanillyl-*trans*-6-nonenamide, Sigma) and resiniferatoxin (RTX, 6,7-Deepoxy-6,7-didehydro-5-deoxy-21-dephenyl-21-(phenylmethyl)daphnetoxin 20-(3-hydroxy-5-methoxybenzeneacetate) from Sigma or from LC Laboratories, USA) were dissolved in ethanol : Tween 80 : physiological saline in the ratio 1:1:8 for a 10 mg/ml capsaicin and a 100 µg/ml RTX stock solution and further dilutions were made with saline. Norepinephrine ((±)-4-(2-Amino-1-hydroxyethyl)-1,2-benzenediol hydrochloride) and oxytocin (α-Hypophamine) from Gedeon Richter, Budapest Hungary) were dissolved in physiological saline.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Statistical analysis

The peak increase or decrease in skin or muscle blood flow (arbitrary units of flux) was determined after drug administration for the assessment of the blood flow. The change of the evoked effect was related to the values measured before drug administration and expressed as percentages (mean ± S.E.M). Changes in arteriolar diameter were expressed as percent change to baseline diameter (constrictions) or in the case of the arteriolar dilations, the percent change of the diameter as was related to the maximal dilation, determined at 80 mmHg intraluminal pressure in a Ca^{2+}-free medium. After normality test the statistical analysis was made by one-way analysis of variance and pairwise multiple comparisons were performed by Student-Newman-Keuls method. The diagrams were plotted using the computer program MicroCal Origin 5.0 (MicroCal Software Inc, Northampton, MA, USA)

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Results

It is well established that vanilloid receptor-1 (TRPV1) mediated vasodilatation involves the activation of TRPV1 in sensory neuronal terminals and the consequent release of neurotransmitters evoking endothelial synthesis of NO (Zygmunt *et al.*, 1999). The effects of TRPV1 stimulation by capsaicin on vascular resistance were tested in the isolated, perfused righ hindlimb of the rat, *in vivo*. Changes in the systemic and perfusion pressure, in the local blood flow in skeletal muscle and in skin of the same hindlimb were measured simultaneously together with the respiration of the rat by pressure transducers and dual channel laser-Doppler flowmetry (Fig. 1A). First, the responsiveness of the perfused rat hindlimb preparations was tested by norepinephrine. Intraarterial injection of norepinephrine (0.5 μg) resulted in an increase of both systemic and tissue blood pressure, a decrease in the blood flow in the perfused skeletal muscle, while no responses were detected in skin perfusion and respiration at this dose (Fig 1). After 15 min regeneration period, the effects of TRPV1 stimulations were tested. Injection of capsaicin (1 μg) into the arterial perfusion cannula of the blood-perfused right hind leg evoked: a decrease in the systemic blood pressure; an increase in the perfusion pressure; a parallel increase in the cutaneous blood flow and a decrease in blood perfusion of the muscle, while respiration was not affected by this dose. These responses were mimicked by the application of the ultrapotent TRPV1 agonist resiniferatoxin (1 μg/kg intravenous injection). In addition, the application of resiniferatoxin resulted in alterations in the respiration and desensitized vanilloid receptors as shown by the unresponsiveness to repeated capsaicin stimulation after 25 min, while the noradrenaline responses were unaltered.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Next, the capsaicin mediated responses were investigated in detail. Intra arterial application of capsaicin (0.1-10 μg) resulted in a dose-dependent increase of perfusion pressure (98±21 mmHg increase at 10 μg, Fig. 1B), an increase in the blood flow in the skin (42±5 AU increase at 3 μg, Fig. 1C) and a decrease in the blood flow in the skeletal muscle (30±4 AU decrease at 3 μg, Fig. 1D).

To determine the role of neural elements in the capsaicin-induced responses, chronic denervation was performed. In a group of animals (n=15) the genitofemoral, femoral and sciatic nerves were crossected 14-20 days before the commencement of the experiment (see Methods). Denervation was not able to abolish capsaicin (1 μg, intra arterial application) evoked rise in the perfusion pressure (Fig. 2, increase in the perfusion pressure is 49±3 mmHg in control and 32±3 mmHg in denervated hindlimbs), nor was it able to affect oxytocin (0.5 I.U.) mediated elevations (49±7 mmHg increase in control and 56±8 mmHg in denervated hindlimbs).

The effect of denervation was also tested on the local blood flow in the skin and in the skeletal muscle (Fig. 3). Capsaicin evoked increase in the blood flow were decreased in the skin (28±3 AU increase in the case of control, versus 18±2 AU in the case of denervated right hindlimb, $P<0.01$, n=7) but the decrease in the blood flow in the skeletal muscle was not affected (9±1.5 AU decrease in the case of control, versus 12±1.5 AU in the case of denervated, $p=0.25$, n=7).

The presence of neurogenic innervation of the arteries in the skin and in the skeletal muscle was tested by immunohistochemistry (Fig. 4). Dense innervation of arteries was found in the skin (filled arrows in Fig. 4), but neurofilament positive nerve terminals were not detected in the skeletal muscle arteries. In contrast, thicker nerves

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

farther away from the vessels were detected in both skin and skeletal muscle tissue samples (labeled by open arrows in Fig. 4).

These results suggested non-neuronal vasoconstriction (decrease in the blood flow) upon TRPV1 stimulation in the skeletal muscle arterioles. To test this hypothesis skeletal muscle (m. gracilis) arteries were isolated and cannulated to directly measure the vasoactive effects of capsaicin. Effects of TRPV1 stimulations were tested after the spontaneous development of the myogenic tone in response to 80 mmHg intraluminal pressure (spontaneous tone: 31±4% of the maximal diameter). TRPV1 activation with capsaicin resulted in a biphasic effect on these arterioles. Low, nanomolar concentrations (0.1-10 nmol/L) of capsaicin resulted in substantial arteriolar dilation (maximum at 10 nmol/L: 32±13%, n=12), which was abolished by the removal of endothelium (Fig. 5A) or by NO synthase inhibition with L-NAME (0.2 mmol/L) (7±5% dilation, n=8 or 1±2% constriction, n=4, respectively). In contrast, higher concentrations of capsaicin (0.1-1 μmol/L) elicited a significant vasoconstriction (Fig. 5A, apparent maximum at 1 μmol/L: 66±7%, n=12), which was not affected by endothelium removal (Fig. 5A, apparent maximum at 1 μmol/L: 68±4%, n=8 after endothelium removal).

We have found that capsaicin-induced arteriolar constriction was transient (Fig. 5B), reaching its maximum at about 90 s (maximal constriction: 59±10%, n=5) and returning to the baseline diameter at the end of the 20 min treatment (3±3% dilation, n=5).

The role of the endogenous activation of TRPV1 on the determination of arteriolar diameter was also tested. Application of the TRPV1 antagonist capsazepine (10

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

μmol/L) resulted in a significant vasodilation (28±7%, n=5) in isolated skeletal muscle arteries.

Based on these functional and immunhistochemical observations we hypothesized that TRPV1 expression is not restricted to sensory neurons in the vasculature. The vascular expression of TRPV1 in the m. gracilis arterioles was evaluated by immunohistochemistry. In these arterioles, TRPV1 expressing cells were co-stained with smooth muscle α-actin (Fig. 6B), demonstrating the expression of TRPV1 in the smooth muscle cells. To further confirm the specificity of the TRPV1 staining, arterioles were co-stained with different TRPV1 antibodies, developed against different TRPV1 epitopes in different species, which resulted in overlapping staining patterns (data not shown). Presence of TRPV1 mRNA in aorta and in cultured A7r5 vascular smooth muscle cells was also confirmed by RT-PCR (Fig. 6A).

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Discussion

The main findings of the present study are that (i) in the hindlimb, TRPV1 stimulation resulted in increase of vascular resistance and reduction of tissue perfusion (Fig. 1), independently of the innervation (Fig. 2 and 3); (ii) in the isolated skeletal muscle arterioles capsaicin elicited vasoconstriction, which was not affected by endothelium removal (Fig. 5); (iii) TRPV1 mRNA is detectable in both aorta and cultured smooth muscle cells (Fig. 6); (iv) TRPV1 is expressed in vascular smooth muscle cells of the skeletal muscle arterioles (Fig. 6). These data suggest functional expression of TRPV1 in the vascular smooth muscle cells of the skeletal muscle arterioles.

Our present findings also indicate that TRPV1 stimulation results in diverse vascular effects in arterial beds of the rat hindlimb. We propose that TRPV1 regulates arteriolar diameter primarily by two mechanisms (Fig. 7). In the case of skin, TRPV1 activation resulted in a neuronal vasodilation (Fig. 1 and 3), probably mediated by a sequence of events such as the activation of sensory neurons, the subsequent local release of sensory neurotransmitters (like CGRP or substance P), stimulation of endothelial cells by these neurotransmitters, activation of endothelial NO synthesis and NO mediated relaxation of smooth muscle cells, as proposed by Zygmunt et al (Zygmunt *et al.*, 1999). In contrast, TRPV1 stimulation by capsaicin had biphasic effects in the isolated skeletal muscle resistance arterioles (vasodilation at lower concentrations and vasoconstriction at higher concentrations, Fig. 5) suggesting a dual regulation of vascular tone. The dilatative effects of capsaicin were endothelium-dependent (Fig. 5) and nitric oxide-mediated. In addition, inhibition of TRPV1 in isolated, pressurized skeletal muscle arteries resulted in a vasodilation, indicating a physiological role of TRPV1 in the regulation of vascular

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

diameter. It should also be noted, that capsaicin evoked opposite effects in different vascular beds under in vivo conditions (Fig. 1A). The increase of pressure of the isovolumetric (3 mL/min) perfusion in the hindlimb suggests higher local resistance in the vasculature of the hindlimb (at least partly as a result of skeletal muscle arteriolar constriction, Fig. 5), while the simultaneous decrease in systemic blood pressure indicates a somewhat higher overall efficiency of vasodilatative receptors (probably mediated by mesenteric, dural, skin, pulmonary or coronary arteries) in the whole vasculature.

The simplest explanation of the findings is that TRPV1 functionally expressed in vascular smooth muscle cells. Although findings based on immunohistochemical data in general should be interpreted with caution, this proposal is also supported by RT-PCR results and the vasoconstrictive effect of TRPV1 stimulation in intact or endothel denuded isolated skeletal muscle arteries. According to these data we hypothesize, that activation of TRPV1 in skeletal muscle arteries occurs both in sensory neurons and in vascular smooth muscle cells, leading to Ca^{2+} influx into both cell types. The elevated intracellular Ca^{2+} concentration in the smooth muscle directly results in vasoconstriction, while in the sensory nerves it triggers neurotransmitter release and concomitant endothel dependent vasodilation (Fig. 7).

Capsaicin evoked *in vivo* vasoconstriction of various arterial beds has been discovered decades ago (Donnerer and Lembeck, 1982;Duckles, 1986;Edvinsson *et al.*, 1990;Molnar and Gyorgy, 1967;Toda *et al.*, 1972). In these initial and the follow up studies, TRPV1 mediated vasoconstriction was found in dog mesenteric (Porszasz *et al.*, 2002;Toda *et al.*, 1972), renal and carotid artery (Toda *et al.*, 1972); in cat middle cerebral (Duckles, 1986;Edvinsson *et al.*, 1990), pial (Edvinsson *et al.*, 1990) and

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

pulmonary (Molnar and Gyorgy, 1967) arteries; in rat heart (Szolcsanyi *et al.*, 2001), small mesenteric (Scotland *et al.*, 2004), dural (Dux *et al.*, 2003) and skeletal muscle arteries (Lizanecz *et al.*, 2006); in mouse knee joint (Keeble and Brain, 2006). Multiple mechanisms leading to TRPV1 mediated vasoconstriction were suggested, including endothelin-1 (Szolcsanyi *et al.*, 2001) or SP (Scotland *et al.*, 2004) release from sensory neurons and yet uncharacterized smooth muscle-mediated effects (Dux *et al.*, 2003;Keeble and Brain, 2006;Porszasz *et al.*, 2002). In addition to these possibilities, our data provide evidence for TRPV1 expression in vascular smooth muscle cells, suggesting a direct link between TRPV1 activation and smooth muscle contraction. Nevertheless, these data indicate that vasoconstrictive effects of TRPV1 stimulation are not restricted to a specific blood vessel, or to a single species.

Interestingly, in some of these cases, like in the case of rat mesenteric arteries both vasoconstriction (Scotland *et al.*, 2004) and vasodilation (Ralevic *et al.*, 2000) were observed upon capsaicin stimulation. It suggests that there are two pools of TRPV1 in these systems, but one of the receptor types are down-regulated under specific circumstances and the physiological effect of capsaicin stimulation is dominated by the active receptor population. In accordance with this idea, dose-dependent biphasic effects were also noted in some studies: low dose capsaicin evoked dilation, while higher concentrations resulted in constriction (Dux *et al.*, 2003;Edvinsson *et al.*, 1990), similarly to our findings.

Several mechanisms have been suggested to regulate TRPV1 activity, besides to the expressional regulation. These include PKC (Bhave *et al.*, 2003) or PKA (Bhave *et al.*, 2002) mediated phosphorylation, calcineurin mediated dephosphorylation (Docherty

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

et al., 1996), interaction with calmodulin (Numazaki *et al.*, 2003) or with phosphoinositides (Liu *et al.*, 2005;Lukacs *et al.*, 2007), besides others. As a matter of TRPV1 mediating skeletal muscle vasoconstriction phosphorylation seems to be the most likely candidate (Lizanecz *et al.*, 2006).

Some of the findings of this study suggest pharmacological differences in the TRPV1 pools mediating constriction and dilation, namely: (i) higher sensitivity/effectivity of dilatative responses (dilation in the case of low capsaicin concentrations), with a profound constrictive responses at maximal stimulation, and (ii) vasodilation evoked by acute inhibition of TRPV1. Additionally, earlier data suggest that the TRPV1 receptors mediating vasodilation can be easily desensitized by neonatal capsaicin treatments, while the capsaicin response of receptors mediating vasoconstriction remains intact or augmented (Donnerer and Lembeck, 1982). As a therapeutical consequence of these observations it seems to be possible to design TRPV1 ligands preferably acting on receptors mediating constrictive or dilatative responses. Although there is no shortage of drug candidates regulating TRPV1 activity (Szallasi *et al.*, 2007), their development was concentrated on their effects on sensory neuronal functions (mostly pain). One of the examples to emphasize the feasibility of such drug development is that it was possible to design an antagonist selective to the plasma membrane located TRPV1 over to the intracellular membrane located receptors (Toth *et al.*, 2004). The drugs selective to receptors mediating dilation or constriction may be useful to regulate blood distribution in various pathophysiological conditions associated with ischemia. For example sensory neuronal TRPV1 was found to be activated upon myocardial ischaemia (Pan and Chen, 2004;Zahner *et al.*, 2003) and beneficial in post

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

ischemic recovery (Wang and Wang, 2005), suggesting that selective activation of sensory neuronal TRPV1 may be beneficial in myocardial infarction.

Taken together, we report here that TRPV1 (a non-specific Ca^{2+} channel) is expressed in smooth muscle cells and its activation leads to vasoconstriction in skeletal muscle resistance arterioles. We propose that TRPV1 has a potential physiological/pharmacological role in the regulation of arteriolar tone in skeletal muscle (apparently in the range of 40% dilation to 60% constriction), which represents a promising new therapeutic strategy to control tissue specific blood distribution.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

References

Akerman S, Kaube H and Goadsby P J (2004) Anandamide Acts As a Vasodilator of Dural Blood Vessels in Vivo by Activating TRPV1 Receptors. *Br J Pharmacol* **142**: 1354-1360.

Bhave G, Hu H J, Glauner K S, Zhu W, Wang H, Brasier D J, Oxford G S and Gereau R W (2003) Protein Kinase C Phosphorylation Sensitizes but Does Not Activate the Capsaicin Receptor Transient Receptor Potential Vanilloid 1 (TRPV1). *Proc Natl Acad Sci U S A* **100**: 12480-12485.

Bhave G, Zhu W, Wang H, Brasier D J, Oxford G S and Gereau R W (2002) CAMP-Dependent Protein Kinase Regulates Desensitization of the Capsaicin Receptor (VR1) by Direct Phosphorylation. *Neuron* **35**: 721-731.

Caterina MJ, Schumacher M A, Tominaga M, Rosen T A, Levine J D and Julius D (1997) The Capsaicin Receptor: a Heat-Activated Ion Channel in the Pain Pathway. *Nature* **389**: 816-824.

Colquhoun EQ, Hettiarachchi M, Ye J M, Richter E A, Hniat A J, Rattigan S and Clark M G (1988) Vasopressin and Angiotensin II Stimulate Oxygen Uptake in the Perfused Rat Hindlimb. *Life Sci* **43**: 1747-1754.

Di Marzo V, Blumberg P M and Szallasi A (2002a) Endovanilloid Signaling in Pain. *Curr Opin Neurobiol* **12**: 372-379.

Di Marzo V, De Petrocellis L, Fezza F, Ligresti A and Bisogno T (2002b) Anandamide Receptors. *Prostag Leukotr Ess* **66**: 377-391.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Docherty RJ, Yeats J C, Bevan S and Boddeke H W (1996) Inhibition of Calcineurin Inhibits the Desensitization of Capsaicin-Evoked Currents in Cultured Dorsal Root Ganglion Neurones From Adult Rats. *Pflugers Arch* **431**: 828-837.

Donnerer J and Lembeck F (1982) Analysis of the Effects of Intravenously Injected Capsaicin in the Rat. *Naunyn Schmiedebergs Arch Pharmacol* **320**: 54-57.

Duckles SP (1986) Effects of Capsaicin on Vascular Smooth Muscle. *Naunyn Schmiedebergs Arch Pharmacol* **333**: 59-64.

Dux M, Santha P and Jancso G (2003) Capsaicin-Sensitive Neurogenic Sensory Vasodilatation in the Dura Mater of the Rat. *Journal of Physiology-London* **552**: 859-867.

Edvinsson L, Jansen I, Kingman T A and McCulloch J (1990) Cerebrovascular Responses to Capsaicin in Vitro and in Situ. *Br J Pharmacol* **100**: 312-318.

Escott KJ and Brain S D (1993) Effect of a Calcitonin Gene-Related Peptide Antagonist (CGRP8-37) on Skin Vasodilatation and Oedema Induced by Stimulation of the Rat Saphenous Nerve. *Br J Pharmacol* **110**: 772-776.

Harris D, McCulloch A I, Kendall D A and Randall M D (2002) Characterization of Vasorelaxant Responses to Anandamide in the Rat Mesenteric Arterial Bed. *J Physiol* **539**: 893-902.

Keeble JE and Brain S D (2006) Capsaicin-Induced Vasoconstriction in the Mouse Knee Joint: a Study Using TRPV1 Knockout Mice. *Neurosci Lett* **401**: 55-58.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Koller A and Bagi Z (2004) Nitric Oxide and H2O2 Contribute to Reactive Dilation of Isolated Coronary Arterioles. *Am J Physiol Heart Circ Physiol* **287**: H2461-H2467.

Liu B, Zhang C and Qin F (2005) Functional Recovery From Desensitization of Vanilloid Receptor TRPV1 Requires Resynthesis of Phosphatidylinositol 4,5-Bisphosphate. *J Neurosci* **25**: 4835-4843.

Lizanecz E, Bagi Z, Pasztor E T, Papp Z, Edes I, Kedei N, Blumberg P M and Toth A (2006) Phosphorylation-Dependent Desensitization by Anandamide of Vanilloid Receptor-1 (TRPV1) Function in Rat Skeletal Muscle Arterioles and in Chinese Hamster Ovary Cells Expressing TRPV1. *Mol Pharmacol* **69**: 1015-1023.

Lukacs V, Thyagarajan B, Varnai P, Balla A, Balla T and Rohacs T (2007) Dual Regulation of TRPV1 by Phosphoinositides. *J Neurosci* **27**: 7070-7080.

Molnar J and Gyorgy L (1967) Pulmonary Hypertensive and Other Haemodynamic Effects of Capsaicin in the Cat. *Eur J Pharmacol* **1**: 86-92.

Numazaki M, Tominaga T, Takeuchi K, Murayama N, Toyooka H and Tominaga M (2003) Structural Determinant of TRPV1 Desensitization Interacts With Calmodulin. *Proc Natl Acad Sci U S A* **100**: 8002-8006.

O'Sullivan SE, Kendall D A and Randall M D (2004) Heterogeneity in the Mechanisms of Vasorelaxation to Anandamide in Resistance and Conduit Rat Mesenteric Arteries. *Br J Pharmacol* **142**: 435-442.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Pan HL and Chen S R (2004) Sensing Tissue Ischemia: Another New Function for Capsaicin Receptors? *Circulation* **110**: 1826-1831.

Porszasz R, Porkolab A, Ferencz A, Pataki T, Szilvassy Z and Szolcsanyi J (2002) Capsaicin-Induced Nonneural Vasoconstriction in Canine Mesenteric Arteries. *Eur J Pharmacol* **441**: 173-175.

Porszasz R and Szolcsanyi J (1994) Antidromic Vasodilatation in the Striated Muscle and Its Sensitivity to Resiniferatoxin in the Rat. *Neurosci Lett* **182**: 267-270.

Ralevic V, Kendall D A, Jerman J C, Middlemiss D N and Smart D (2001) Cannabinoid Activation of Recombinant and Endogenous Vanilloid Receptors. *Eur J Pharmacol* **424**: 211-219.

Ralevic V, Kendall D A, Randall M D, Zygmunt P M, Movahed P and Hogestatt E D (2000) Vanilloid Receptors on Capsaicin-Sensitive Sensory Nerves Mediate Relaxation to Methanandamide in the Rat Isolated Mesenteric Arterial Bed and Small Mesenteric Arteries. *Br J Pharmacol* **130**: 1483-1488.

Ross RA (2003) Anandamide and Vanilloid TRPV1 Receptors. *Br J Pharmacol* **140**: 790-801.

Santha P and Jancso G (2003) Transganglionic Transport of Choleragenoid by Capsaicin-Sensitive C-Fibre Afferents to the Substantia Gelatinosa of the Spinal Dorsal Horn After Peripheral Nerve Section. *Neuroscience* **116**: 621-627.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Scotland RS, Chauhan S, Davis C, De Felipe C, Hunt S, Kabir J, Kotsonis P, Oh U and Ahluwalia A (2004) Vanilloid Receptor TRPV1, Sensory C-Fibers, and Vascular Autoregulation: a Novel Mechanism Involved in Myogenic Constriction. *Circ Res* **95**: 1027-1034.

Szallasi A and Blumberg P M (1999) Vanilloid (Capsaicin) Receptors and Mechanisms. *Pharmacol Rev* **51**: 159-211.

Szallasi A, Cortright D N, Blum C A and Eid S R (2007) The Vanilloid Receptor TRPV1: 10 Years From Channel Cloning to Antagonist Proof-of-Concept. *Nat Rev Drug Discov* **6**: 357-372.

Szolcsanyi J (1988) Antidromic Vasodilatation and Neurogenic Inflammation. *Agents Actions* **23**: 4-11.

Szolcsanyi J, Oroszi G, Nemeth J, Szilvassy Z, Blasig I E and Tosaki A (2001) Functional and Biochemical Evidence for Capsaicin-Induced Neural Endothelin Release in Isolated Working Rat Heart. *Eur J Pharmacol* **419**: 215-221.

Toda N, Usui H, Nishino N and Fujiwara M (1972) Cardiovascular Effects of Capsaicin in Dogs and Rabbits. *J Pharmacol Exp Ther* **181**: 512-521.

Toth A, Blumberg P M, Chen Z and Kozikowski A P (2004) Design of a High-Affinity Competitive Antagonist of the Vanilloid Receptor Selective for the Calcium Entry-Linked Receptor Population. *Mol Pharmacol* **65**: 282-291.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Wang L and Wang D H (2005) TRPV1 Gene Knockout Impairs Postischemic Recovery in Isolated Perfused Heart in Mice. *Circulation* **112**: 3617-3623.

Zahner MR, Li D P, Chen S R and Pan H L (2003) Cardiac Vanilloid Receptor 1-Expressing Afferent Nerves and Their Role in the Cardiogenic Sympathetic Reflex in Rats. *J Physiol* **551**: 515-523.

Zygmunt PM, Petersson J, Andersson D A, Chuang H, Sorgard M, Di M, V, Julius D and Hogestatt E D (1999) Vanilloid Receptors on Sensory Nerves Mediate the Vasodilator Action of Anandamide. *Nature* **400**: 452-457.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Footnotes

Sources of funding: The study was supported by the Hungarian Academy of Sciences OTKA (F048873 and K68077 to AT, T046244 to RP and F-048837 to ZB) and by the Hungarian Ministry of Health (ETT 430/2006 to AT, ETT 493/2006 to RP, ETT-454-2006 to IE) grants. Z.B., Z.P., R.P. and A.T. were supported by Bolyai Fellowship of the Hungarian Academy of Sciences.

Reprint requests may be addressed to:

Attila Tóth, PhD

Division of Clinical Physiology, Institute of Cardiology, Medical and Health Science Center, University of Debrecen

22 Moricz Zs krt, 4032, Debrecen, Hungary

Tel.: (36) 52-414-928

Fax: (36) 52-323-978

E-mail: atitoth@dote.hu

[1] These authors served as co-senior authors

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Legends for Figures

Fig. 1 Effects of TRPV1 stimulation in perfused hindlimb of the rat

The left common carotid artery and the common iliac artery were cannulated for blood perfusion of the right hind limb of the rat (3 mL/min). Systemic (arterial blood pressure and respiration) and local (perfusion pressure, blood flow in the skin and skeletal muscle) effects of noradrenaline (NA, 0.5 μg i.a.), Capsaicin (Caps, 1 μg i.a.) and resiniferatoxin (RTX, 1 μg/kg i.v.) were recorded on the same preparation. A representative of the *in vivo* experiments performed is shown in panel A. In the following panels the dose-response of capsaicin is shown on the perfusion pressure (B), on the blood flow of the skin (C) and skeletal muscle (D) on the right hind limb. Values are average±S.E.M.

Fig. 2 Effects of denervation on the perfusion pressure changes of the rat right hindlimb, *in vivo*

In a group of animals (n= 15) the nerves innervating the right hind leg were transected 7-20 days before the measurement. The effects of capsaicin (1 μg i.a.) and oxytocin (0.5 I.U.) were tested on the perfusion pressure in control and in denervated hind limbs. Values are average±S.E.M.

Fig. 3 Effects of denervation on the blood flow changes of the rat right hind limb, *in vivo*

Denervation was performed as mentioned earlier. The effects of capsaicin (1 μg i.a.) on the blood flow of skin and skeletal muscle were tested in control and denervated hind limbs. Values are average±S.E.M., n=7.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 4 Innervation of arteries in the skin and skeletal muscle of the rat hind limb

The skin (paw) and skeletal muscle (m. gracilis) of the hindlimb were sectioned in a cryostate (thickness 10 μm) and fixed in acetone. The presence of neuronal elements (innervation) was tested by a neurofilament specific antibody (Sigma, dilution: 1:100) and visualized by a secondary antibody conjugated with Texas red (red on the figures). For the staining of the arteries, a smooth muscle specific antibody (Novocastra, dilution: 1:20) and a secondary antibody conjugated with Cy-2 (green on the figures) were used. Localization of these elements were visualized in cross and longitudinal sections. Thick nerves in the tissue sections are represented by open arrows, while thin neurits innervating the arteries are shown by closed arrows.

Fig. 5 Effects of TRPV1 stimulation in isolated arterioles

Dose-response of capsaicin on isolated pressurized skeletal muscle arterioles (control, n=12 and endothelium denuded n=8) are shown in panel A. The effect of 20 min continuous application of 1 μmol/L capsaicin is shown in panel B. Values are average±S.E.M.

Fig. 6 Expression of TRPV1 in vascular smooth muscle cells

RT-PCR analysis was performed using RNA isolated from aorta and cultured vascular smooth muscle cells (panel A). TRPV1 specific primers were designed to yield a 149 bp fragment from mRNA (shown by the arrow) and a 596 bp fragment from DNA (not detected). Glycerol aldehyde phosphate dehydrogenase (GAPDH) was used as control. To investigate the localization of TRPV1 immunohistochemistry was performed in the

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Molecular Pharmacology Fast Forward. Published on February 6, 2008 as DOI: 10.1124/mol.107.043323
This article has not been copyedited and formatted. The final version may differ from this version.

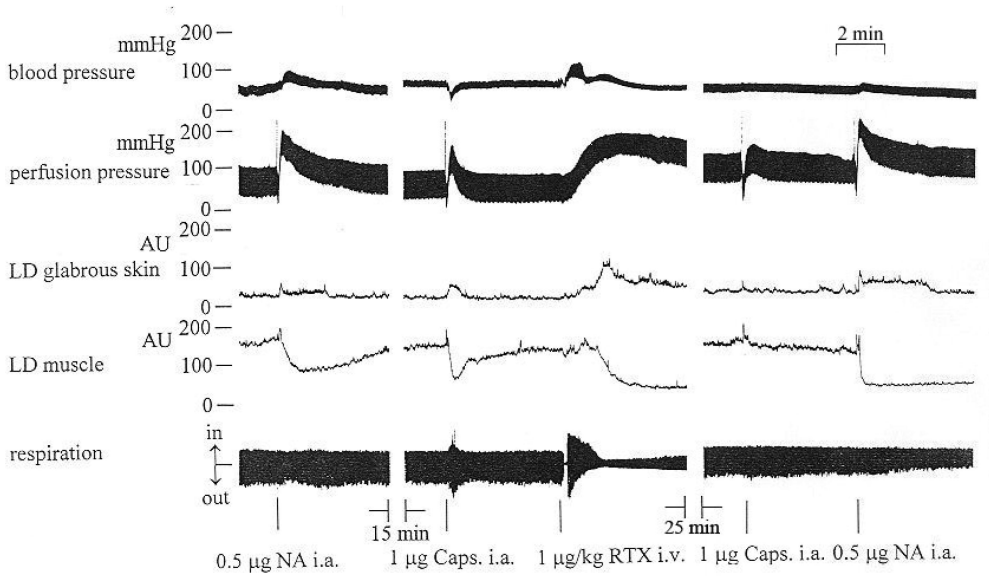
skeletal muscle (m. gracilis) tissue sections of the rat (panel B). TRPV1 (rabbit, Calbiochem, 1:500 dilution, green) and smooth muscle actin (monoclonal, Novocastra, 1:20 dilution, red) specific staining was visualized by a fluorescent microscope. Slides were also processed in mounting media containing DAPI for staining of nuclei (blue). The artery used in the *in vitro* functional studies is identified (L: lumen, A: artery).

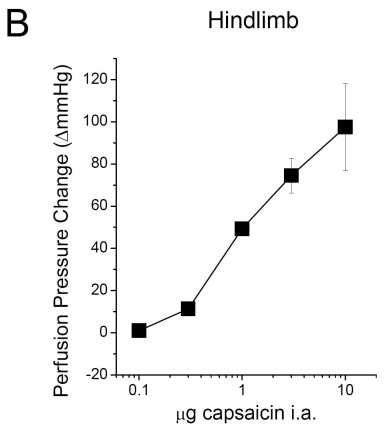
Fig. 7 Proposed mechanism of tissue specific regulation of vascular diameter by TRPV1

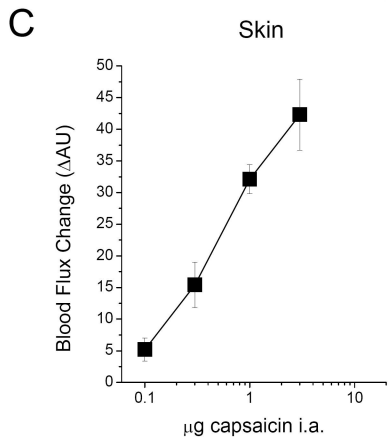
Our data support the well known sensory neuronal dilation in the skin arteries, involving the following events: (i) activation of sensory neuronal TRPV1; (ii) elevation of intracellular Ca^{2+} in the neuronal terminals; (iii) release of sensory neurotransmitters, including calcitonin gene related peptide (CSRP) and substance P (SP); (iv) activation of endothelial receptors of these neurotransmitters; (v) increase of endothelial nitric monoxide (NO) synthesis; (vi) NO diffusion and smooth muscle relaxation. The same mechanism was found to be responsible for capsaicin mediated dilation in skeletal muscle arteries. In contrast, functional expression of TRPV1 was also identified in vascular smooth muscle cells of m. gracilis. The activation of these smooth muscle located receptors led to vasoconstriction. We propose that the balance of activities of sensory neuronal and smooth muscle located TRPV1 mediated pathways determines the vasoactive effects of TRPV1 stimulation.

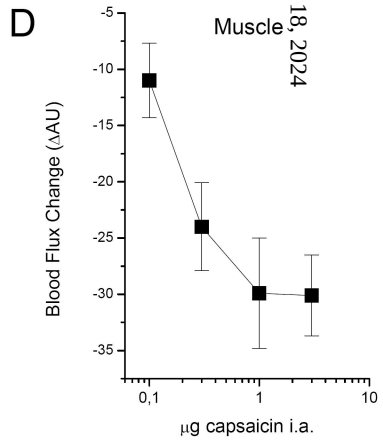
Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Fig. 1 A



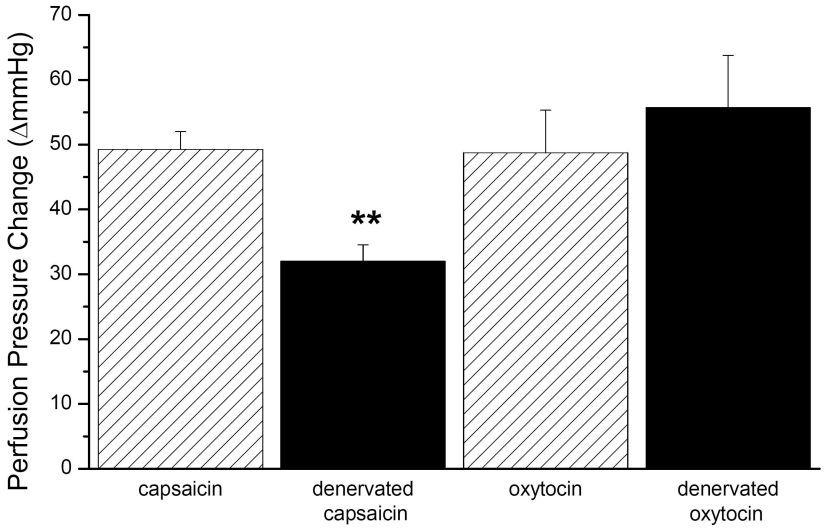






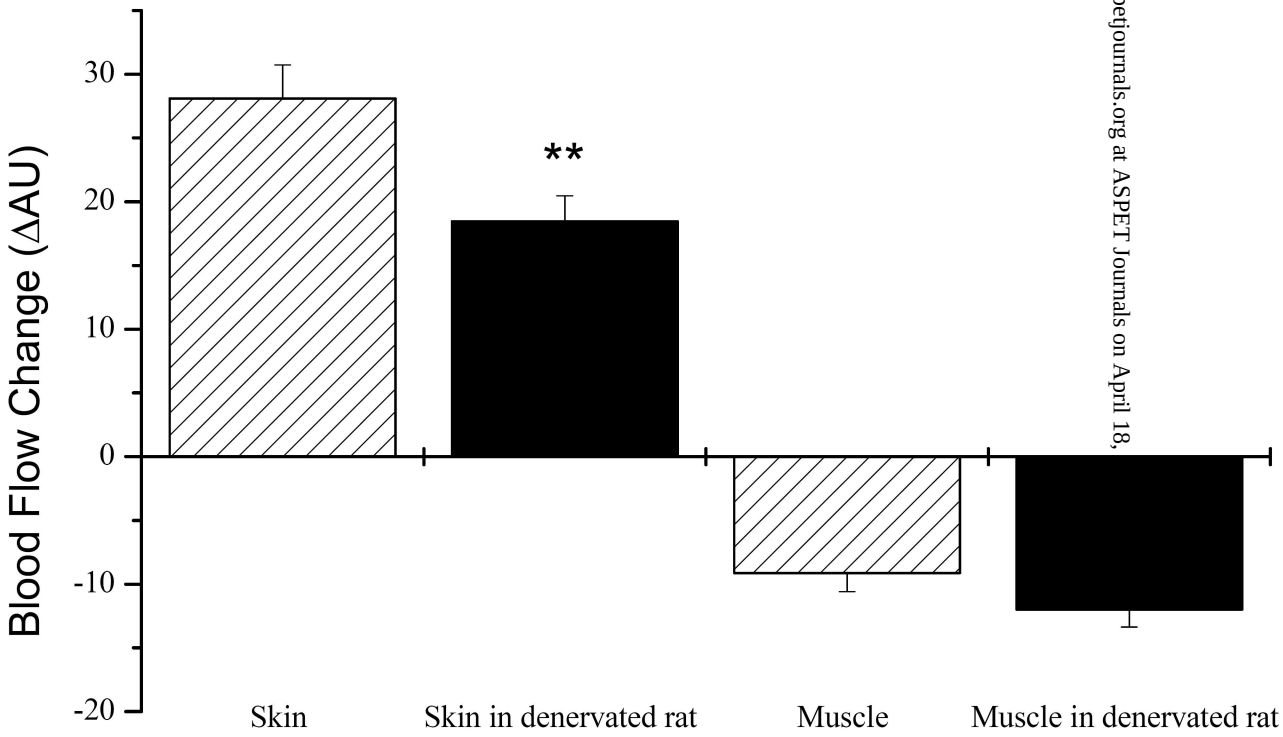
Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Fig. 2



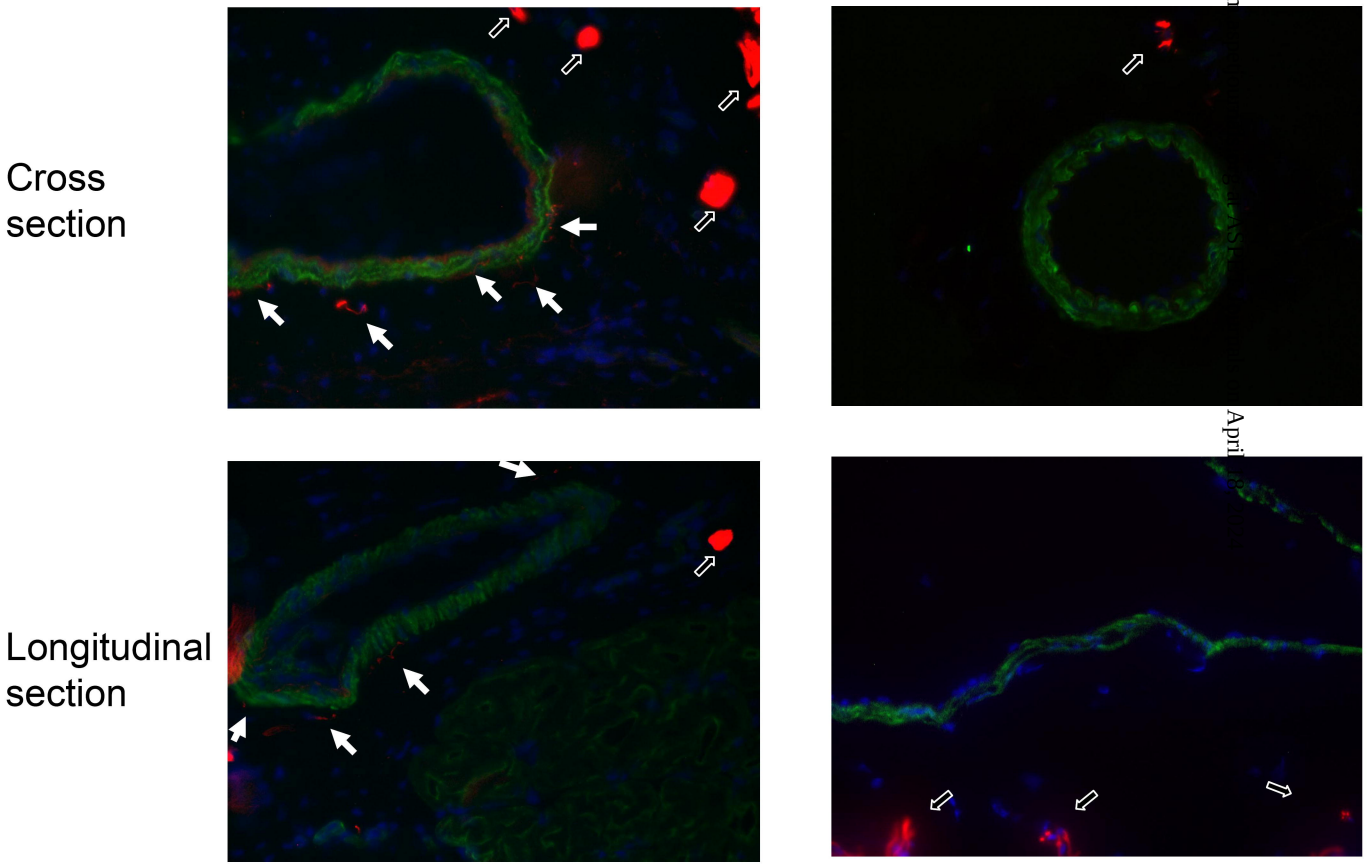
Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Fig. 3



Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18,

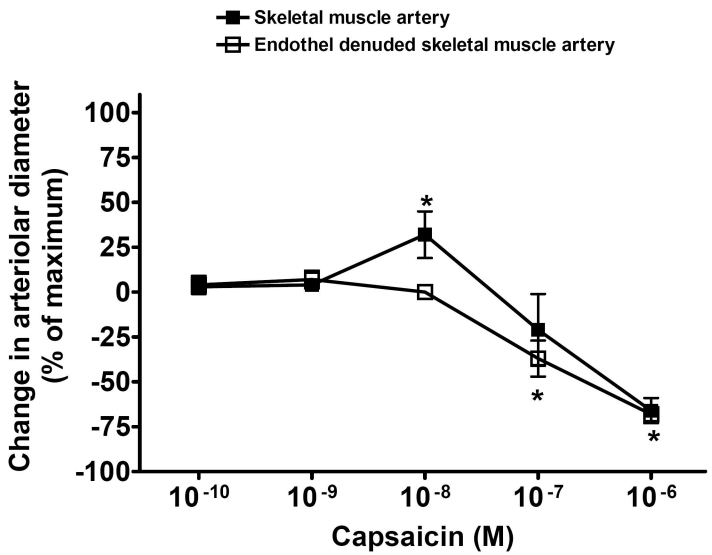
Fig. 4



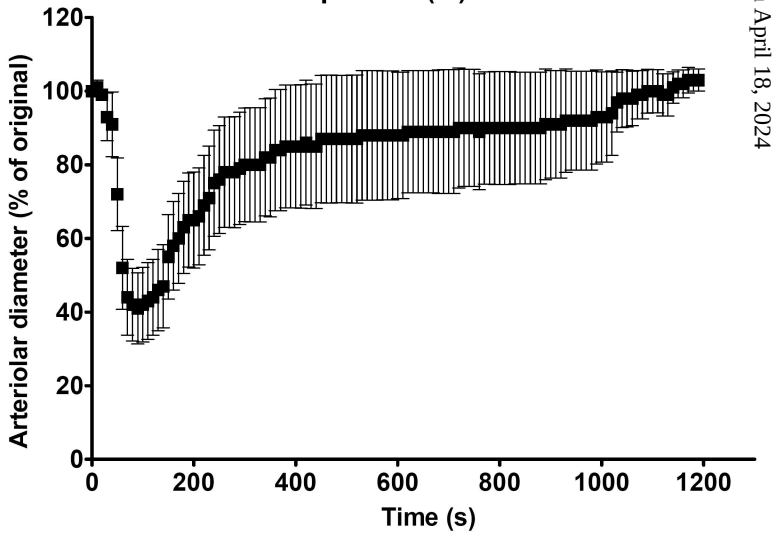
Downloaded from molpharm

Fig. 5

A

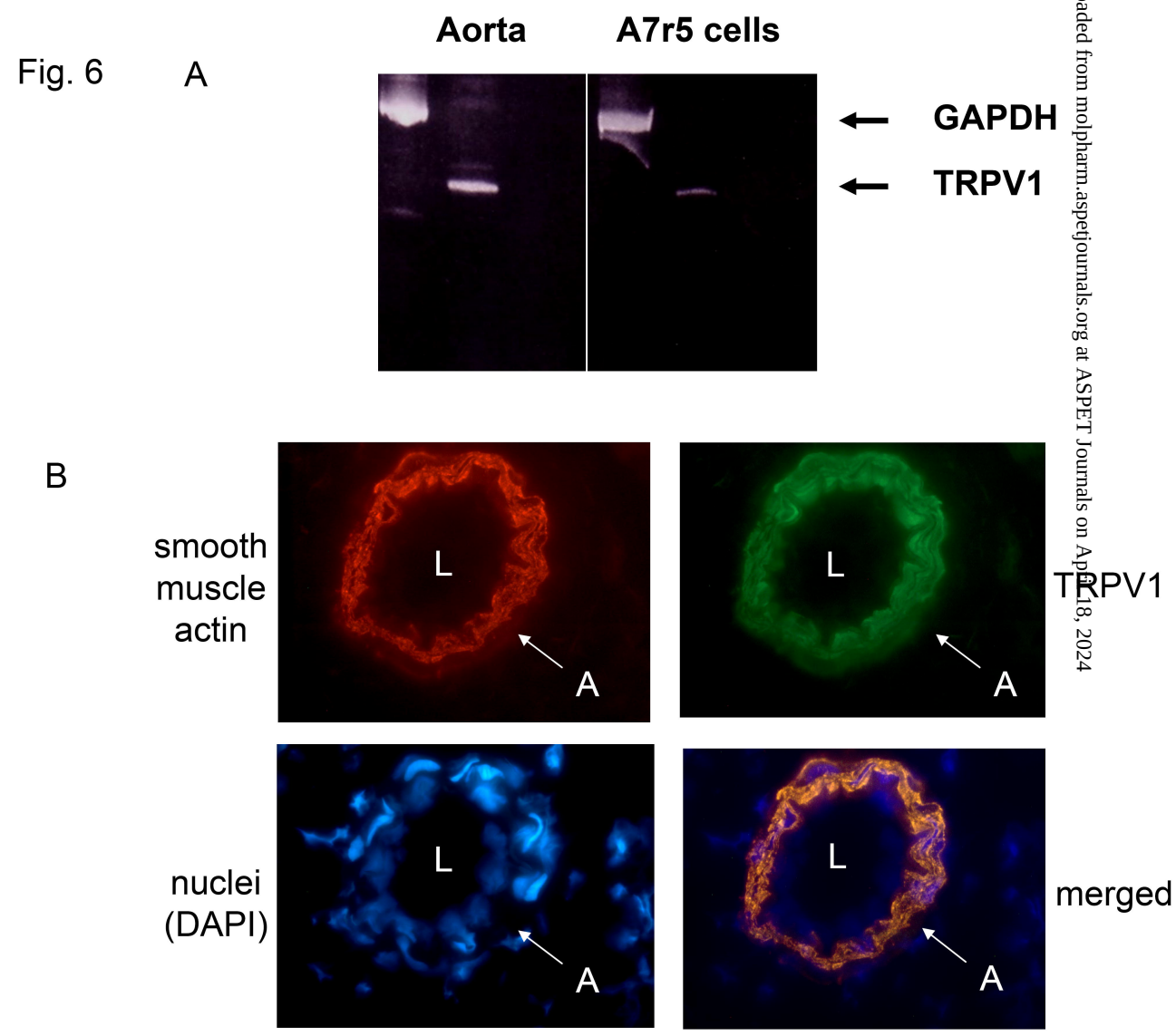


B



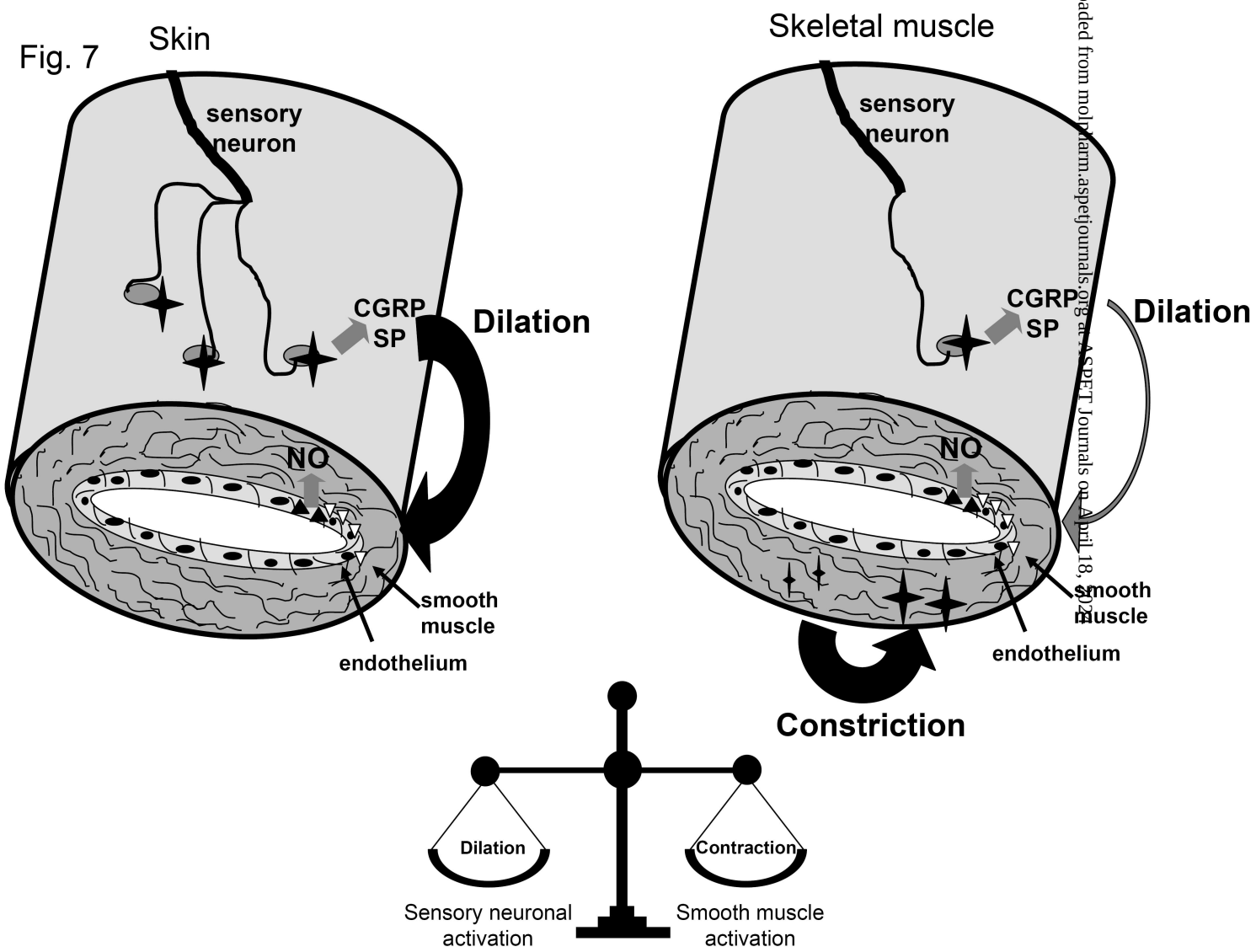
Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Fig. 6



Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

Fig. 7



Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024