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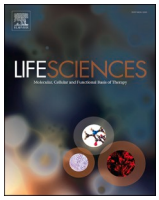

Evidence for the involvement of TRPV2 channels in the modulation of vascular tone in the mouse aorta

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ABSTRACT

Aims: Transient receptor potential vanilloid 2 (TRPV2) channels are expressed in both smooth muscle and endothelial cells and participate in vascular mechanotransduction and sensing of high temperatures and lipids. Nevertheless, the impact of TRPV2 channel activation by agonists on the coordinated and cell-type specific modulation of vasoreactivity is unknown.

Main methods: Aorta from 2- to 4-months-old male Oncins France 1 mice was dissected and mounted in tissue baths for isometric tension measurements. TRPV2 channel expression was assessed by immunofluorescence and western blot in mice aortas and in cultured A7r5 rat aortic smooth muscle cells.

Key findings: TRPV2 channels were expressed in all three mouse aorta layers. Activation of TRPV2 channels with probenecid evoked endothelium-dependent relaxations through a mechanism that involved activation of smooth muscle K_{ir} and K_v channels. In addition, TRPV2 channel inhibition with tranilast increased endothelium-independent relaxations to probenecid and this effect was abrogated by the K_{ATP} channel blocker glibenclamide, revealing that smooth muscle TRPV2 channels induce negative feedback on probenecid relaxations mediated *via* K_{ATP} channel inhibition. Exposure to the NO donor sodium nitroprusside increased TRPV2 channel translocation to the plasma membrane in cultured smooth muscle cells and enhanced negative feedback on probenecid relaxations.

Significance: In conclusion, we present the first evidence that TRPV2 channels may modulate vascular tone through a balance of opposed inputs from the endothelium and the smooth muscle leading to net vasodilation. The fact that TRPV2 channel-induced activity can be amplified by NO emphasizes the pathophysiological relevance of these findings.

1. Introduction

Transient receptor potential (TRP) channels are a set of membrane proteins that act as non-selective cation channels [1–3]. The TRP vanilloid (TRPV) channel subfamily (TRPV1–6) is located mostly in the plasma membrane and is expressed in different cell types and tissues [4]. Like other TRPV channels, TRPV2 are formed by a long cytoplasmic N-terminal domain, followed by six transmembrane segments containing a

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loop that forms the pore, and the cytoplasmic C-terminal domain [5]. To date, it has been reported that these channels are activated by high temperatures (>52 °C), mechanical stress, and various chemical signals [5–8]. TRPV2 channels are ubiquitously expressed in the body and have been detected in smooth muscle cells [9–11], endothelial cells [12], and in the heart [13]. This evidence suggests that these channels may have widespread roles in cardiovascular physiology [5,14].

TRPV2 channels are essential for the maintenance of cardiac structure and function [15,16] and play a critical role in dystrophic cardiomyopathy [17] and myocardial infarction [18]. Regarding the involvement of TRPV2 in physiological or pathological vascular responses, there is insufficient knowledge. In the few studies that reported the participation of TRPV2 channels in vascular physiology, the authors showed that these channels could act as sensors of membrane stretch and cell swelling in mouse aortic smooth muscle cells [11]. Likewise, other studies have proved that myogenic responses in retinal arterioles are initiated by pressure-induced stretching, which activates TRPV2 channels [19]. Altogether, available evidence suggests that TRPV2 channels play important roles in vascular physiology. Nevertheless, there is scarce knowledge about the impact of these channels on the coordinated and cell-type specific modulation of vasoreactivity.

Up to now, one of the main limitations of the study of TRPV2 channel functions is the lack of ligands that specifically interact with them. Certain molecules such as some growth factors, cannabinoids, lysophospholipids, insulin, 2-aminoethoxydiphenyl borate (2-APB), nitric oxide (NO), and cytokines activate TRPV2 channels-derived responses [5–7,20]. Some of them induce translocation of TRPV2 channels from the endoplasmic reticulum to the plasma membrane (*e.g.*, NO), while others can act as direct modulators of channel gating. For instance, cannabidiol and 2-APB activate TRPV2 channels directly, but they can also activate other channels and receptors [21]. In addition, this activity could be species-dependent since human TRPV2 channels are insensitive to 2-APB [22]. Interestingly, the uricosuric agent probenecid, which shows antihypertensive effects in rats [23], has also been recognized as a TRPV2 channel activator [24–27], whereas the antiallergic drug tranilast has been used as a TRPV2 channel antagonist [28–30]. Nevertheless, both drugs have also been considered to interact with other targets [5,7].

The involvement of smooth muscle and/or endothelial TRPV2 channel signaling in vascular responses is not explored. The present study was designed to investigate the putative participation of endothelial and smooth muscle TRPV2 channels in regulating vascular reactivity of the mouse aorta, using probenecid and tranilast as pharmacological tools.

2. Materials and methods

2.1. Animals

Two- to four-months old male mice ($n = 64$) of the Oncins France 1 (OF1) strain were used (Charles River, Sant Cugat del Vallès, Spain). Preliminary data showed that probenecid responses were comparable within this range of age. The animals were housed in accordance with the regulations of the institutional guidelines: constant temperature and humidity, light-dark cycle of 12–12 h and no limit of food or water. All procedures were carried out following the Spanish Legislation (RD35/2013) and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication number 85–23, revised in 1996). Likewise, the procedure was approved by the Ethics Committee of the Universitat Autònoma de Barcelona (approval code: FJA-eut/01).

2.2. Preparation of aortic rings

Mice were euthanized by rapid decapitation under anesthesia (4 % isoflurane mixed with 0.8 L/min O_2). With the aim of gently dissecting the thoracic and abdominal aorta, the thoracic cavity of the mouse was opened, and the lungs and heart were removed. Immediately after dissection, the aorta was placed in a cold oxygenated (95 % O_2, 5 % CO_2) Krebs Henseleit (KH) physiological solution. While the artery was immersed in the cold KH solution, the connective and adipose tissue was gently removed and the artery thoracic ascendent, arch, thoracic descendent, and abdominal portions were dissected.

2.3. Evaluation of aortic functionality

Aortic segments (1.5 mm) were mounted under isometric conditions on a 4-channel wire myograph (Model 620 M; Danish Myo Technology, Aarhus, Denmark) with cold KH solution [31]. Next, the aortic segments were incubated for 30 min at 37 °C in oxygenated (95 % O_2, 5 % CO_2) KH solution. Once stabilization was complete, the arteries were gradually stretched until the basal ring tension reached the optimal tension of 14.7 mN. After a 45 min stabilization period, tissue viability was analyzed by exposing the segments twice to a K^+-rich KH solution (100 mM KCl). Subsequently, a series of washes were carried out and the segments were again subjected to a stabilization period of 30 min. The stable analogue of thromboxane A2, 9,11-Dideoxy-9α,11α-methaneepoxy prostaglandin F 2α (U46619; Merck KGaA, Darmstadt, Germany) was added to the bath at a concentration to induce a pre-contraction of 70–100 % compared to the contraction produced by the 100 mM KCl solution. Once a plateau was reached, increasing concentrations of acetylcholine (ACh; from 1 nM up to 100 μM) were added to assess endothelium-dependent relaxations. Those aortic rings that showed <50 % relaxation were discarded, as they were considered to have damaged endothelium. Subsequently, a series of washes were performed and after a stabilization period of 30 min aortic segments were pre-contracted with U46619 to then perform a concentration-response curve (from 1 nM to 300 μM) using the agonist of TRPV2 channels, probenecid. Probenecid relaxations in the presence of pre-contractions to phenylephrine (Phe), an α_1-adrenergic receptor agonist, or 5-hydroxytryptamine (5-HT), a 5-HT receptor agonist, were also studied. Preliminary studies showed no sex-dependent differences in probenecid relaxations in aortas from C57BL/6 mice (unpublished results); consequently, only male mice were used.

To analyze probenecid mechanisms of action we used the abdominal aorta, since relaxations were prominent in this aortic portion and four segments per animal could be regularly obtained. We evaluated the effect produced by several inhibitors that were pre-incubated 30 min before the probenecid responses began. Tranilast (100 μM), a TRPV2 channel inhibitor, glibenclamide (30 μM), an ATP-dependent K^+ (K_{ATP}) channels blocker, 4-aminopyridine (1 mM), an inhibitor of voltage-gated K^+ (K_v) channels, apamin (100 nM) and charybdotoxin (100 nM), an inhibitor of Ca^{2+}-activated K^+ channels, barium chloride ($BaCl_2$; 100 μM), an inhibitor of inwardly rectifying K^+ (K_{ir}) channels, prazosin (0.1 μM), a selective α1-adrenergic receptor blocker, and sodium nitroprusside (NPS; 100 μM), a NO donor.

Finally, with the aim of establishing the contribution of endothelium in probenecid relaxations, the vessels were pre-incubated for 30 min with NG-nitro-L-arginine methyl ester (L-NAME; 300 μM), a non-selective inhibitor of synthases of NO, and with indomethacin (10 μM), a non-selective inhibitor of cyclooxygenases. In addition, the endothelium was removed mechanically by gently rubbing the interior of the vessel with a plastic cannula. Arteries with ACh relaxations of <20 % were considered endothelium denuded. In endothelium-denuded aortas, probenecid relaxations were tested in the absence and presence of $BaCl_2$ (100 μM) plus 4-aminopyridine (1 mM) or tranilast (100 μM) in different experimental conditions.

2.4. Cell culture

The rat aortic smooth muscle cell line A7r5 was obtained from ECACC (European Collection of Cell Cultures) and cultured in high

glucose Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich) supplemented with 10 % fetal bovine serum (FBS). Cells were maintained at 37 °C in a humidified atmosphere containing 5 % CO_2. Cells were seeded at 40,000 cells/mL on collagen-coated (collagen 1 rat tail, BD Biosciences) coverslips in case of immunofluorescences, grown for 48 h and then starved in 0.2 % FBS medium for 1 h before adding treatments. NPS was freshly prepared in 0.2 % FBS DMEM, protected from light, and used at 10, 100 and 300 μM final concentrations for 1 h.

2.5. Immunofluorescence

Aortic segments were fixed in 4 % paraformaldehyde and embedded in Tissue Tek OCT embedding medium (Sakura Finetek Europe, Zoeterwoude, the Netherlands), frozen in liquid nitrogen and kept at −70 °C. Sections were processed for immunofluorescence staining as previously described [32]. Briefly, 14 μm-thick frozen transverse sections were incubated for 1 h with anti-TRPV2 (1:100; #ACC-039, lot ACC039AN0502, Alomone Labs, Jerusalem, Israel) primary polyclonal antibody in a humidified chamber at 37 °C. Afterwards, sections were incubated for 45 min with a donkey anti-rabbit IgG secondary antibody conjugated to Cyanine 3 (1:200; #711-165-152, lot 110351, Jackson ImmunoResearch Laboratories, West Grove, PA, USA) in a humidified chamber at 37 °C. The specificity of the immunostaining was verified by omission of the primary antibody (negative control). Images were captured using a FV1000 confocal microscope (Olympus Iberia, Barcelona, Spain). Average fluorescence signal was analyzed in at least two rings of each animal with Image J software (National Institutes of Health, Bethesda, MD, USA), and the results were expressed as arbitrary units.

In the case of cultured smooth muscle cells, after the corresponding treatments, they were washed once with phosphate-buffered saline (PBS) containing 0.5 mM Ca^{2+} chloride ($CaCl_2$), 1 mM magnesium chloride ($MgCl_2$) and 4 % sucrose (PBS-Ca-Mg-Suc). Cells were then incubated for 5 min at 37 °C with anti-TRPV2 (1/60; #ACC-039, lot ACC039AN0502, Alomone Labs, Jerusalem, Israel) in PBS-Ca-Mg-Suc. After a wash in ice-cold PBS-Ca-Mg, cells were fixed in PBS-Ca-Mg-Suc containing 2 % paraformaldehyde for 15 min on ice. Then, cells were washed twice in PBS-Ca-Mg and blocked in PBS containing 2 % normal goat serum (NGS) for 20 min at 37 °C under slow agitation. After one wash with PBS-Ca-Mg, cells were incubated with Cy3-conjugated donkey anti-rabbit IgG secondary antibody (1:250 in PBS containing 2 % NGS) for 1 h at 37 °C under slow agitation. Finally, after two 10 min washes in PBS, coverslips were mounted using Vectashield plus containing DAPI (#H-2000-2) to counterstain nuclei (Vector Laboratories, Burlingame, CA, USA) and images were captured using a FV1000 confocal microscope (Olympus Iberia, Barcelona, Spain). Average fluorescence signal per area was analyzed in at least two regions per experimental condition with ImageJ-FIJI software (National Institutes of Health, Bethesda, MD, USA), and the results were expressed as arbitrary units.

2.6. Biotinylation and subcellular fractionation

To obtain surface-protein fractions, a biotinylation protocol was performed on cultured smooth muscle cells, as previously described [33]. Briefly, cells were rinsed in ice-cold PBS-Ca-Mg and then incubated for 30 min at 4 °C under slow agitation in 0.5 mg/mL of biotin diluted in the same buffer (EZ-Link Sulfo-NHS-SS-Biotin; Thermo Fisher Scientific). Then, cells were washed three times in PBS-Ca-Mg containing 100 mM Glycine, once in PBS-Ca-Mg, and scrapped in lysis buffer (50 mM NaCl, 10 mM EDTA, 10 mM EGTA, 50 mM HEPES, 1 % Triton X-100, and protease and phosphatase inhibitors) to obtain lysates. These were incubated overnight at 4 °C on rotation with NeutrAvidin-agarose beads (Thermo Fisher Scientific), reserving part of the lysate as Total Fraction. After centrifugation at 4000 ×*g* for 4 min at 4 °C, the supernatant was recovered and stored as internal fraction, and beads were washed four times in lysis buffer and reconstituted in sample buffer, boiled for 5 min at 95 °C and centrifuged to obtain the membrane fraction. Samples were analyzed by western blot.

2.7. Western blot

The protein concentration of samples was determined by the Bradford method (Bio-Rad, Hercules, CA, USA). Equal amounts of protein were loaded for the total and internal fractions (15 to 25 μg, depending on the experiment), while equal volumes of the membrane fractions were loaded between treatments (40 to 50 μL). Proteins were separated by SDS-polyacrylamide gel electrophoresis using the Bio-Rad Mini-PROTEAN 3 system and transferred onto nitrocellulose membranes (Schleicher & Schüll). Membranes were blocked for 1 h with PBS containing 0.1 % Tween-20 and 10 % non-fat dry milk and then incubated overnight at 4 °C with the indicated primary antibody diluted in blocking buffer. After incubation with the corresponding HRP (horseradish)-conjugated secondary antibodies, blots were developed using the EZ-ECL Enhanced Chemiluminescence Detection Kit for HRP (Biological Industries, Kibbutz Beit Haemek, Israel) and captured with Fuji X-ray films Super RX-N (Fujifilm, Tokyo, Japan). The antibodies used were: rabbit anti-TRPV2 (1:500; #ACC-039, lot ACC039AN0502, Alomone Labs, Jerusalem, Israel); mouse anti-GAPDH (1:10000; Enzo Life Sciences); rabbit anti-insulin-like growth factor 1 receptor b (IGFRecB; 1:1000; Santa Cruz Biotechnology, Dallas, TX, USA), HRP-conjugated goat anti-rabbit IgG (1:2000; Sigma-Aldrich), HRP-conjugated rabbit anti-mouse IgG (1:2000; Sigma-Aldrich). Band intensities were quantified with ImageJ-FIJI software (National Institutes of Health, Bethesda, MD, USA), and normalized with the Ponceau staining corresponding to each lane. GAPDH and IGFRecB were used as controls for the correct fractionation protocol in internal and membrane fractions, respectively.

2.8. High-performance liquid chromatography (HPLC)

The concentration of ATP in mice aortas was evaluated by HPLC with ultraviolet detection. A pool of thoracic plus abdominal aorta segments from 2 animals was used until reaching $n = 5$ independent experiments per experimental condition (10 mice). The tissue was homogenized with 60 μL of modified RIPA buffer (50 mM Tris-HCl, 0.5 % sodium deoxycholate, 0.2 % SDS, 100 mM NaCl, 1 mM EDTA, 1 % Triton X-100) with a douncer. The method used for ATP extraction from biological samples was adapted from Sudo et al. (2000) [34]. After incubating at 37 °C for 20 min, samples were centrifuged, and 25 μL of supernatant was injected into the HPLC system (Perkin Elmer series 200 Pump, 717 plus Auto sampler, 2487 Dual λ absorbance detector). A reverse-phase silica-based chromatography column (Kromasil 100-5-c18; 4.0 × 200 mm) was obtained from Teknokroma (Sant Cugat del Vallès, Spain). The mobile phase was methanol-10 mM tetrabutylammonium hydrogen sulfate in 20 mM ammonium dihydrogen phosphate-diammonium hydrogen phosphate (pH 6.5) (25:75), which was run with an isocratic regular flow rate of 1.0 mL/min and the wavelength UV detector was set at 260 nm.

2.9. Drugs and reagents

U46619 (#D8174), ACh (#A6625), phenylephrine (#P6126), serotonin (#H9523), probenecid (#P8761), tranilast (#T0318), 4-aminopyridine (#A78403), glibenclamide (#60639), L-NAME (#N5751), indomethacin (#I7378), NPS (#71778), $BaCl_2$ (#B0750), prazosin (#P7791), carbenoxolone (#C4790), and reagents used to make solutions were purchased from Merck (KGaA, Darmstadt, Germany). Apamin (#L8407) and charybdotoxin (#L8213) were acquired from Latoxan (Portes-lès-Valence, France). Acetylcholine, probenecid, 4-aminopyridine, $BaCl_2$, apamin, charybdotoxin, and carbenoxolone were dissolved in distilled water, while tranilast, and stock solutions (10 mM) of glibenclamide and U46619 were dissolved in DMSO. We ensured that

the highest concentration of DMSO in the bath did not exceed 0.005 %. L-NAME and NPS were dissolved in KH solution. Mounting medium containing DAPI was obtained from Vectashield. Reagents used for biotinylation assay were obtained from ThermoFisher Scientific.

2.10. Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM) of the number of animals (n) in each group (shown in figure legends). Normal distribution was checked before choosing the appropriate statistical analysis. Comparison between two groups was carried out with the Mann-Whitney U or Student's t-test. Comparison between more than two groups (one single factor) was carried out with one-way ANOVA or the Kruskal-Wallis test followed by Tukey's or Dunn's post-test, respectively. The vasodilator responses induced by ACh and probenecid were expressed as % of the relaxation obtained after precontraction with U46619, Phe, 5-HT, or with a 100 mM KCl solution. Statistical analysis was performed using GraphPad Prism software version 9.1 (GraphPad Software, San Diego, California, USA). Two-way/factor ANOVA tests were performed with repeated measures on the concentration factor and using the Bonferroni or Tukey's post-test. In some graphs, the area under the curve was calculated and expressed as arbitrary units of the number of animals (n) in each group (shown in figure legends). In this case, the statistical analysis consisted of an ordinary two-way ANOVA test. Statistical significance was considered based on a value of $P < 0.05$.

3. Results

3.1. TRPV2 channels are expressed in the different layers of the mouse aortic wall

Immunofluorescent expression of TRPV2 channels was assessed in mouse aortic cross sections (Fig. 1). TRPV2 channel signal was detected in all three (adventitia, media, and endothelial) layers of the vessel wall. Nevertheless, in the case of endothelial and smooth muscle cells only, the signal was widely distributed throughout the cell body, whereas TRPV2 channel expression in adventitial cells was in the nucleus.

3.2. TRPV2 channels modulate vascular tone in the mouse aorta

Probenecid, an agonist of TRPV2 channels, shows antihypertensive effects in rats, which have been associated with the blockade of aortic contraction [23]. However, the involvement of TRPV2 channels in this vascular activity has not been previously analyzed. Fig. 2A shows concentration-dependent relaxations to this TRPV2 channel agonist in different aortic regions previously pre-contracted with U46619. Relaxations were similar in the ascending aorta, the aortic arch, and the abdominal aorta, whereas the thoracic descending aorta showed significantly lower responses. At this stage, we continued our study in the abdominal aorta, since it showed high probenecid relaxations, while allowing four segments per animal to be obtained consistently for comparative studies.

Previous studies in Phe-precontracted rat [23] and human [35] arteries suggested that probenecid-induced vasodilatations could be directly or indirectly related to inhibition of α-adrenergic receptors. In our experimental conditions, probenecid relaxations were not likely to be mediated *via* blockade of α_1-adrenergic receptor signaling, since they were induced in aortas previously pre-contracted with Phe, but also with other agents. In fact, in the abdominal aortic portion, probenecid relaxations were higher in U46619 and 5-HT pre-contracted vessels *versus* other contractile agents such as Phe and KCl (100 mM) (Fig. 2B). We therefore studied probenecid relaxations in aortas pre-contracted with U46619 in posterior mechanistic studies. Moreover, the selective α_1-adrenergic receptor blocker prazosin 0.1 μM did not relax mouse aortas pre-contracted with U46619 (Supplementary Fig. 1), thus, adding further evidence against participation of α_1-adrenoceptors in mouse aorta probenecid relaxations.

It is remarkable that the relaxant activity of probenecid was almost indetectable in aortic rings pre-contracted with KCl, suggesting an intervention of K^+ channels in this activity, which will be analyzed later (section 3.3).

Compared to ACh relaxations, an endothelium-dependent muscarinic receptor agonist used as a reference, probenecid concentration-responses curves shifted to the right in all aortic segments, indicating lower relaxations (Supplementary Fig. 2). However, maximum relaxations evoked by probenecid and ACh were not statistically different, except for thoracic descending aorta, which showed lower relaxations (Supplementary Fig. 2).

Participation of the endothelium in probenecid vasorelaxant activity was shown by comparing its activity in intact and endothelium-denuded vessels. Fig. 3A shows a significant decrease of probenecid-induced relaxation in aortas without endothelium. However, after endothelium removal, remaining probenecid-induced relaxations (24.30 ± 4.21 %) could still be observed. The non-selective inhibition of NO synthase (Fig. 3B) and COX (Fig. 3C) isoforms with L-NAME (300 μM) or indomethacin (10 μM), respectively, did not modify probenecid relaxations, excluding participation of these endothelial mediators in the vasorelaxant response of probenecid. These findings demonstrate that aortic relaxations to probenecid have both endothelium-dependent and independent components.

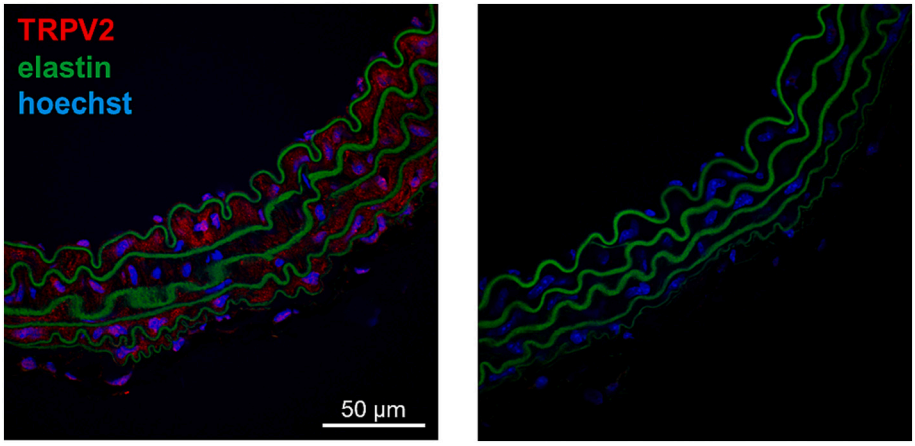

Fig. 1. Expression of TRPV2 channels in the mouse aorta. Representative photomicrographs of TRPV2 channel immunofluorescence (red; left) and negative control (right) of confocal microscopic mouse abdominal aorta sections ($n = 5$). Natural autofluorescence of elastin (green) and nuclear staining with Hoechst 33342 (10 μg/mL) (blue) are also shown.

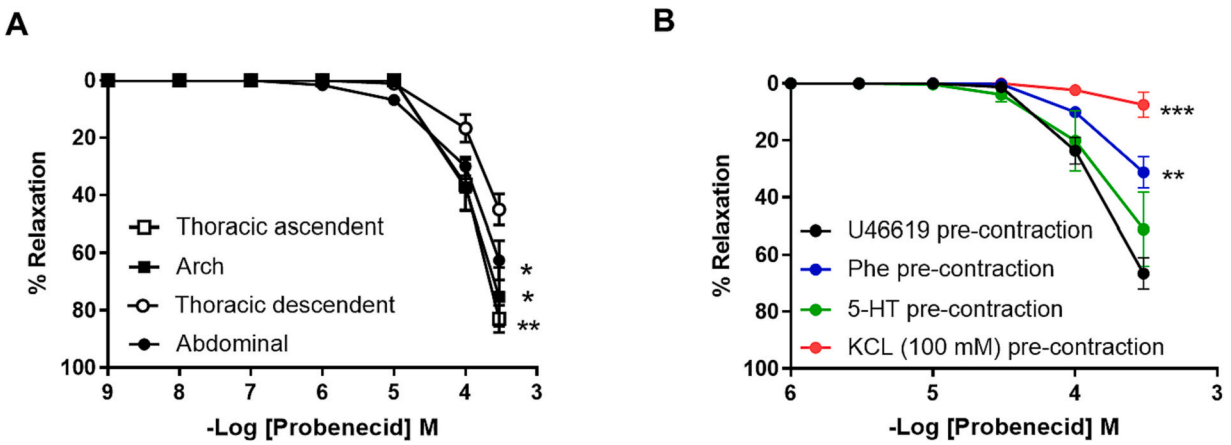

Fig. 2. Effects of the aortic region or the pre-contractile agonist on probenecid relaxations. (A) Concentration-response curves to probenecid in U46619 pre-contracted aortas from different aortic regions. (B) Concentration-response curves to probenecid in U46619, phenylephrine (Phe), 5-hydroxytryptamine (5-HT), and KCl pre-contracted abdominal aorta. Results are the mean ± SEM from 4 to 5 (A) and 4–5 (B) mice. $^{*}P < 0.05$, $^{**}P < 0.01$ *versus* thoracic descendent (A); $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* U46619 pre-contraction (B) by two-way ANOVA.

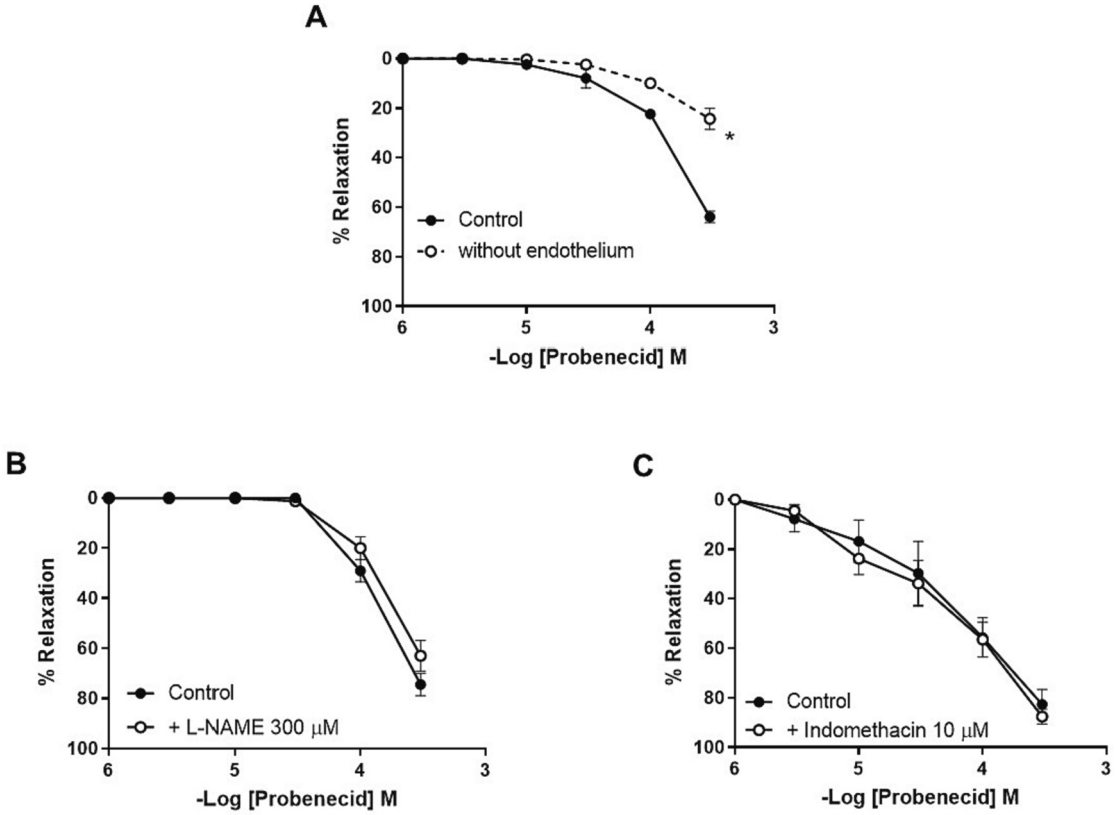

Fig. 3. Modulation of probenecid relaxations by the endothelium. (A) Concentration-response curves to probenecid in aortic rings with and without endothelium. (B) Effect of the non-selective NO synthase inhibitor, L-NAME (300 μM), on the concentration-response curves to probenecid in U46619 pre-contracted abdominal aortas. (C) Effect of the non-selective cyclooxygenase inhibitor, indomethacin (10 μM), on the concentration-response curves to probenecid in U46619 pre-contracted abdominal aortas. Results are the mean ± SEM from 6 to 7 (A), 5 (B), and 5–8 (C) mice. $^{*}P < 0.05$ by two-way ANOVA.

We then used the TRPV2 channel blocker tranilast (100 μM) [28–30] to determine the role of TRPV2 channels in probenecid responses. Incubation with tranilast significantly increased probenecid (Fig. 4A) but not ACh (Fig. 4B) relaxations in endothelium-intact aortas. The potentiation effect of tranilast on probenecid relaxations was also observed in endothelium-denuded arteries (Fig. 4A) suggesting that, in our experimental conditions, tranilast antagonizes TRPV2 channels located out of the endothelial layer. The fact that tranilast did not modify ACh-induced relaxations excludes an unspecific activity of TRPV2 channels as modulators of vascular tone and ascribes this activity to a specific antagonist activity of tranilast at the probenecid-activated smooth muscle TRPV2 channels. These results suggest the presence of negative feedback on probenecid relaxations induced by muscular TRPV2 channels.

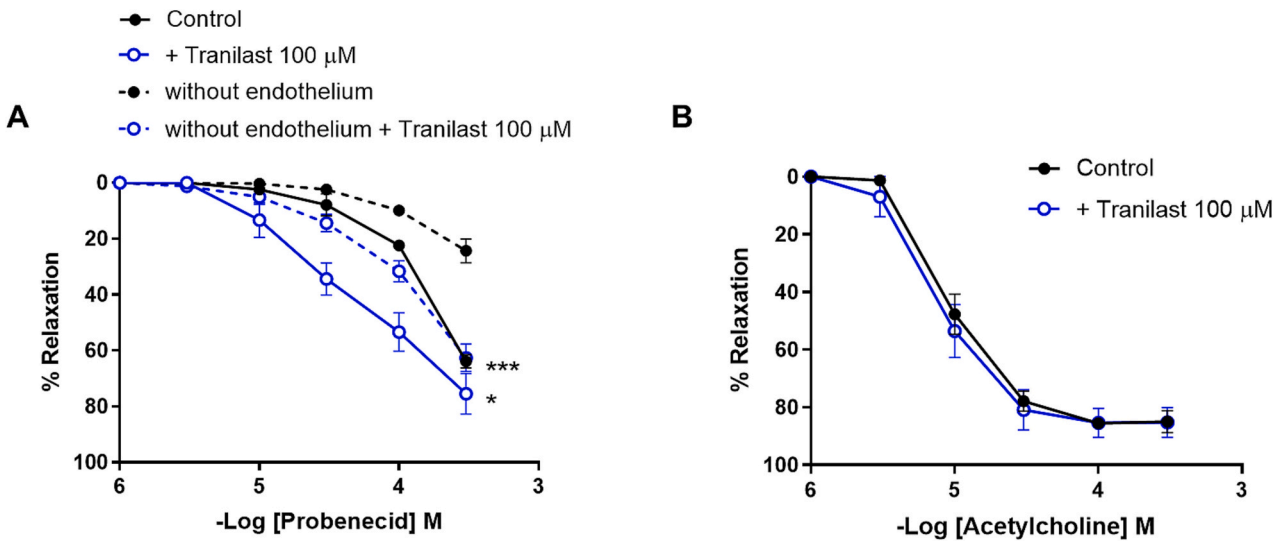

Fig. 4. Impact of inhibition of TRPV2 channels on probenecid or acetylcholine relaxations. (A) Effect of the selective TRPV2 inhibitor, tranilast (100 μM), on the concentration-response curves to probenecid in U46619 pre-contracted abdominal aortas with or without endothelium. (B) Effect of tranilast (100 μM) on the concentration-response curves to acetylcholine in U46619 pre-contracted abdominal aortas. Results are the mean ± SEM from 5 to 9 (A) and 4 (B) mice. $^{*}P < 0.05$ *versus* control, $^{***}P < 0.001$ *versus* without endothelium by two-way ANOVA.

3.3. *Probenecid-induced endothelium-dependent relaxations are mediated by K_{IR} and K_V channels*

As reported above (Fig. 2B), probenecid relaxations were abolished in KCl (100 mM)-pre-contracted arteries, which suggest the involvement of smooth muscle hyperpolarization due to K^+ efflux through K^+ channels. Thus, concentration-response curves to probenecid were performed in vessels incubated with different K^+ channel inhibitors and

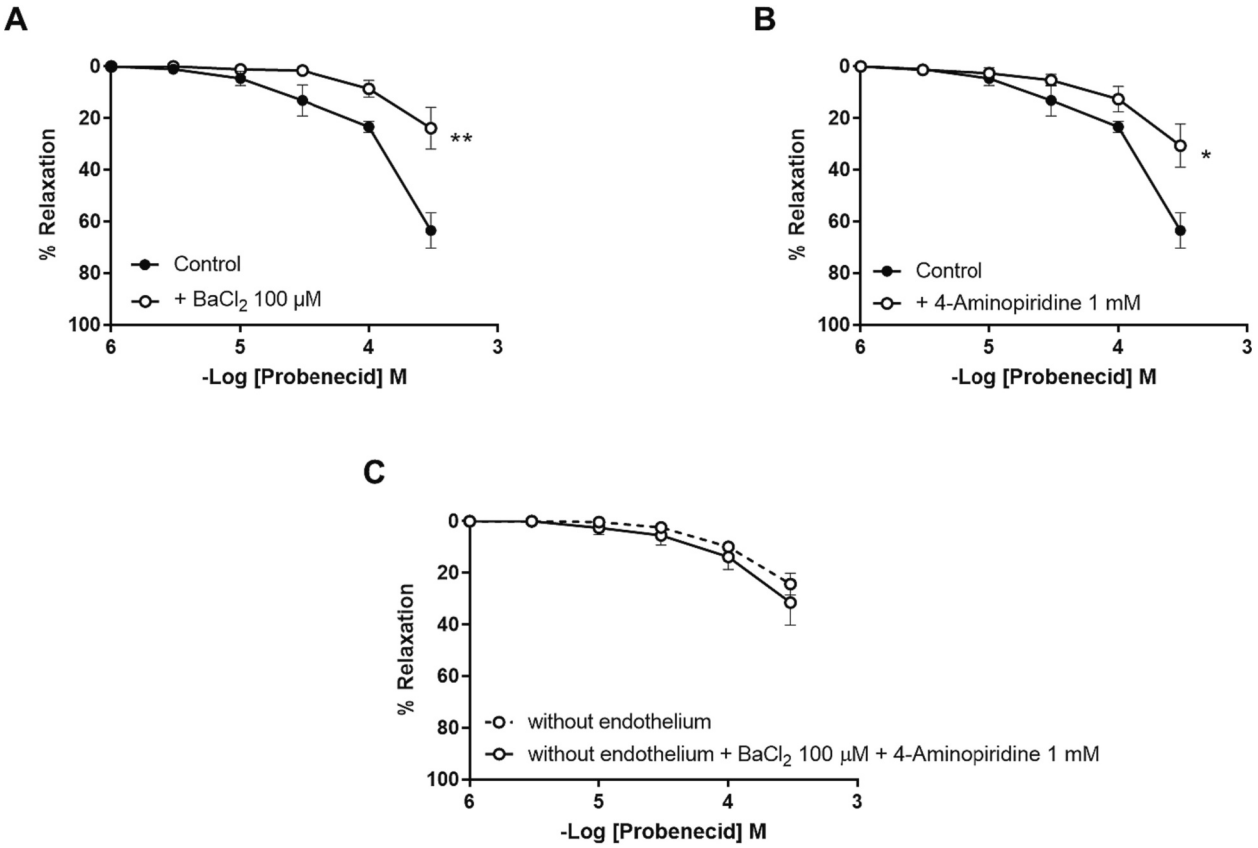

Fig. 5. Effects of inhibition of K^+ channels on probenecid relaxations. (A) Concentration-response curves to probenecid in U46619 pre-contracted abdominal aortas in the absence (control) or presence of the inwardly rectifying K^+ channel blocker $BaCl_2$ (100 μM). (B) Concentration-response curves to probenecid in U46619 pre-contracted abdominal aortas in the absence (control) or presence of the voltage-gated K^+ channel blocker 4-aminopyridine (1 mM). (C) Concentration-response curves to probenecid in U46619 pre-contracted abdominal aortas without endothelium in the absence or presence of $BaCl_2$ plus 4-aminopyridine. Results are mean ± SEM from 4 to 5 (A), 4–5 (B), and 6 (C) mice. $^{*}P < 0.05$, $^{**}P < 0.01$ by two-way ANOVA.

with an inhibitor of the Na^+K^+-ATPase. Neither inhibition of K_{Ca} channels with charybdotoxin (100 nM) and apamin (100 nM), nor inhibition of K_{ATP} channels with glibenclamide (30 μM) modified probenecid relaxations (Supplementary Fig. 3). In addition, blockade of Na^+K^+-ATPase-mediated hyperpolarization with ouabain (1 μM) did not have an effect (Supplementary Fig. 3). In contrast, either inhibition of K_{IR} channels with $BaCl_2$ (1 mM) (Fig. 5A) or K_V channels blockade with 4-aminopiridine (1 mM) (Fig. 5B) significantly reduced probenecid relaxations in aortic rings with endothelium. Probenecid relaxations obtained in aortas without endothelium were not affected by incubation with $BaCl_2$ (1 mM) plus 4-aminopiridine (1 mM) (Fig. 5C). Altogether, these results suggest that endothelium-dependent probenecid relaxations are mediated by K_{IR} and K_V channels.

3.4. Activation of muscular TRPV2 channels by probenecid favors ATP accumulation with blockade of K_{ATP} channels

We then investigated the mechanism involved in the TRPV2 channel-mediated modulation of endothelium-independent probenecid relaxations, which was revealed after the blockade of TRPV2 channels with tranilast. The experiments were performed in aortas without endothelium to facilitate the interpretation of results.

In the presence of the TRPV2 channel antagonist tranilast, neither incubation of $BaCl_2$ (1 mM), nor charybdotoxin (100 nM) and apamin (100 nM), nor 4-aminopiridine (1 mM), nor ouabain (1 μM) modified probenecid responses (Supplementary Fig. 4). In contrast, incubation with the K_{ATP} channel inhibitor glibenclamide (30 μM) prevented endothelium-independent probenecid-induced relaxations evidenced by TRPV2 channel blockade with tranilast (Fig. 6A). Taken together, these results suggest that K_{ATP} channels are involved in the potentiation effect of tranilast on probenecid endothelium-independent relaxations. We hypothesized that activation of muscular TRPV2 channels by probenecid favors ATP accumulation with blockade of K_{ATP} channels, depolarization, opening of voltage-dependent Ca^{2+} channels and increase in the contractile tone, which opposes probenecid relaxations. Consistently, aortic intracellular ATP concentrations in the presence of probenecid were significantly decreased by TRPV2 channel blockade with tranilast (Fig. 6B). However, carbenoxolone, an inhibitor of pannexin-1 channels, did not modify the decrease in ATP concentrations elicited by probenecid (Fig. 6B), excluding the participation of these channels in this vascular response.

3.5. Nitric oxide induces the translocation of TRPV2 channels to the plasma membrane and increases the tranilast-induced potentiation of probenecid relaxations

Previous studies showed that TRPV2 channels translocate from the endoplasmic reticulum to the plasma membrane in response to NO stimulation [20]. To elucidate whether exposure to NO translocates TRPV2 channels to the plasma membrane in vascular cells, we cultured A7r5 rat aortic smooth muscle cells in the absence and presence of the NO donor NPS, and measured TRPV2 channel location to the membrane by immunofluorescence and biotinylation of surface proteins followed by subcellular fractionation and western blot. Immunofluorescence was performed with primary antibodies incubated in alive cultured cells, allowing only the extracellularly exposed TRPV2 channels to bind. In non-treated cells, TRPV2 channel fluorescence was distributed in heterogeneous clusters located at the plasma membrane (Fig. 7A). Incubation with NPS (300 μM) tended ($P = 0.070$) to increase the amount of TRPV2 channel-derived clusters of fluorescence (Fig. 7A), suggesting an elevated presence of the receptor in the cellular membrane. To confirm that this phenomenon was a result of the translocation of TRPV2 channels to the plasma membrane, biotinylation of cell surface proteins followed by subcellular fractionation was performed and then analyzed by western blot. In biotinylated fractions of smooth muscle cells, corresponding to proteins facing outside the plasma membrane, exposure to NPS (100 μM) promoted an increased presence of TRPV2 channels in the membrane fraction of the cells (Fig. 7B). In endothelium-denuded aortas, incubation with NPS (100 μM) did not modify probenecid relaxations, but significantly increased the tranilast-induced potentiation of these responses (Fig. 8).

4. Discussion

TRPV2 channels are expressed in smooth muscle and endothelial cells of some vascular beds and participate in vascular mechanotransduction and sensing of high temperatures and lipids [5–8]. Nevertheless, the mechanisms associated with TRPV2 channel activation by agonists on the fine-tune modulation of vascular tone are unknown. Here, for the first time, we demonstrate that the TRPV2 channel agonist probenecid causes endothelium-dependent and independent relaxations, which are counteracted by TRPV2 channels located at the muscular layer. Endothelium-dependent relaxations induced by probenecid are mediated through activation of K_{ir} and K_v channels.

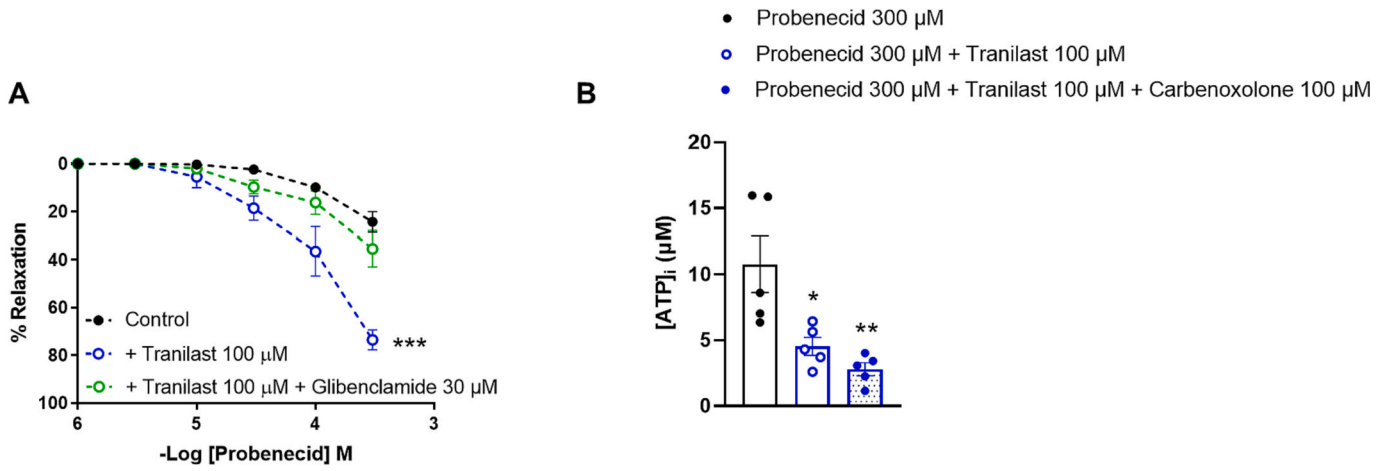

Fig. 6. Impact of inhibition of K_{ATP} channels on the effect of tranilast on probenecid relaxations in abdominal aortas without endothelium. (A) Concentration-response curves to probenecid in U46619 pre-contracted abdominal aortas in the absence (control) or presence of tranilast (100 μM) and the KATP channel blocker glibenclamide (30 μM). (B) Intracellular concentrations of ATP measured by HPLC in abdominal aortas exposed to probenecid (300 μM) and treated with tranilast, or tranilast plus carbenoxolone (100 μM). Results are mean ± SEM from 5 to 10 (A) mice and 5 independent experiments (10 mice) (B). ***$P < 0.001$ *versus* control by two-way ANOVA (A); *$P < 0.05$; **$P < 0.01$ *versus* Probenecid by one-way ANOVA (B).

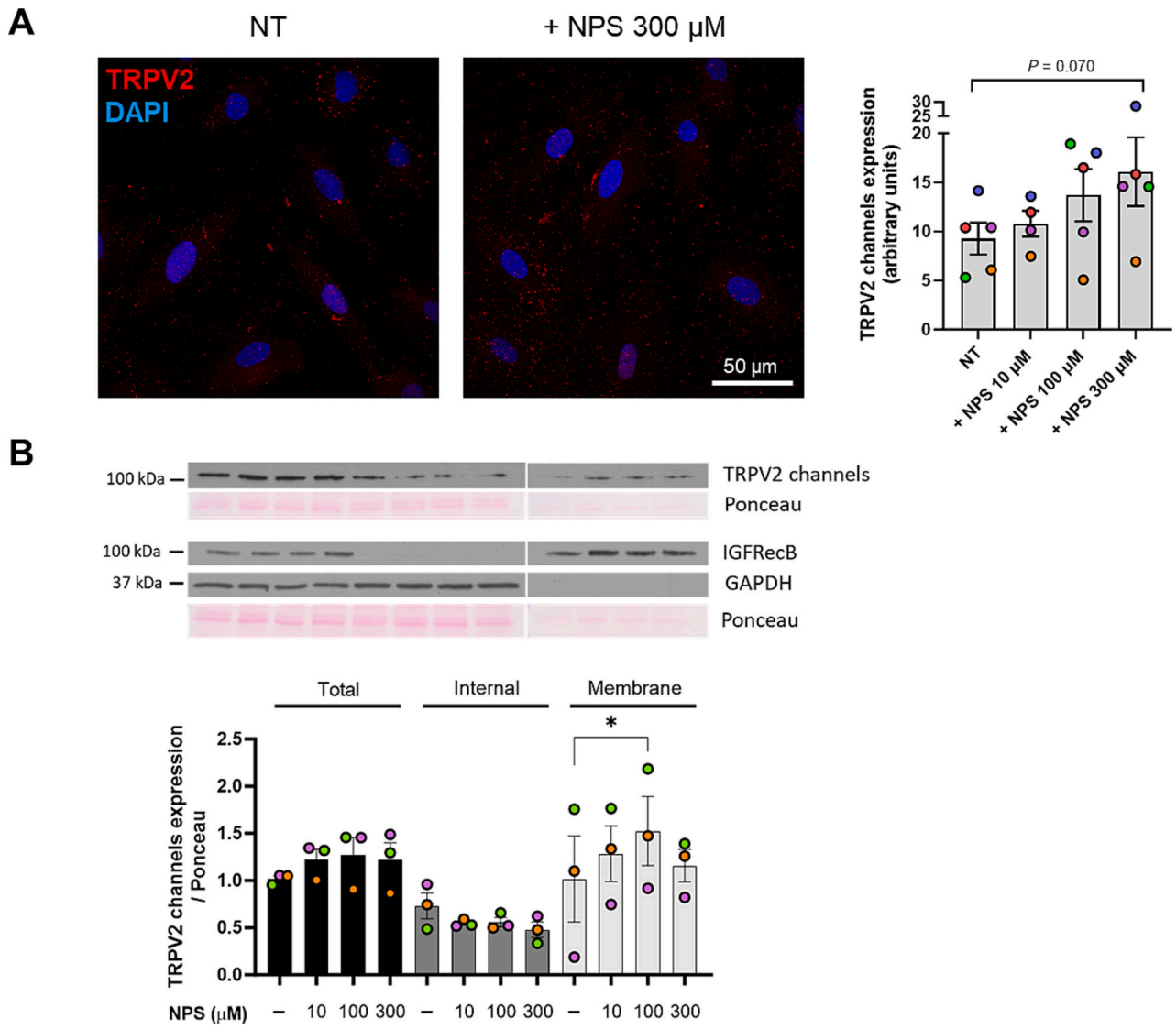

Fig. 7. Impact of NO exposure on TRPV2 channel location in the plasma membrane. (A) Representative photomicrographs of TRPV2 channel immunofluorescence (red; left) and quantification (right) of fluorescence (area x intensity) in non-treated (NT) and treated (sodium nitroprusside, NPS, 10–300 μM) A7r5 rat aortic smooth muscle cells. Nuclear staining with DAPI (blue) is also shown. (B) Western blot detection of TRPV2 channels in total, internal and membrane fractions obtained after cell surface biotinylation and subsequent subcellular fractionation in NT and treated (NPS, 10–300 μM) A7r5 rat aortic smooth muscle cells (above), with the corresponding signal quantification (below). Controls of membrane and internal fractions are represented by IGFRecB and GAPDH, respectively. Ponceau staining was used as a loading control. Results are the mean ± SEM from 5 (A) and 3 (B) independent experiments. The colored dots indicate values of each independent experiment. $P = 0.070$ by one-way ANOVA; $^*P < 0.05$ by paired Student's *t*-test.

Endothelium-independent relaxations are more evident when TRPV2 channels are blocked by tranilast, demonstrating that TRPV2 channels located out of the endothelium exert negative feedback on probenecid relaxations. We propose that smooth muscle TRPV2 channels activated by probenecid trigger an increase in ATP levels with the consequent K_{ATP} channel inhibition (Fig. 9). Furthermore, we show that NO augments the translocation of TRPV2 channels to the plasma membrane of aortic smooth muscle cells, an effect that is associated with an increase in the negative feedback on probenecid relaxations (Fig. 10).

Previous studies showed that TRPV2 channels are expressed at the transcriptional level in rat aortas without endothelium [9]. In addition, these channels were also detected in human pulmonary artery endothelial cells [12]. In the present study, we show that TRPV2 channels are expressed in endothelial, smooth muscle and adventitial cells of the mouse aorta, and then we study their potential functional role in modulating aortic tone. We demonstrate that the TRPV2 channel agonist probenecid evokes aortic relaxations, largely attenuated by endothelium removal. It is worth noting that after the removal of this layer, probenecid still evoked endothelium-independent relaxations, as discussed later.

In a previous study in rat thoracic aortas pre-contracted with the α_1-adrenergic receptor agonist Phe, the authors reported that endothelium removal was insufficient to block probenecid-induced relaxations, which were attributed to α-adrenergic receptor blockade [23]. In our conditions, the classic α_1-adrenoceptor antagonist prazosin was unable to relax U44619 pre-contracted mouse aortas, suggesting that this signaling pathway is not likely to participate in our experimental conditions. Although we do not know the reasons for the discrepancy in the mechanisms involved in probenecid relaxations between our study and previous evidence [23], we speculate that the use of a different pre-contractile agonist (U46619 *versus* Phe), lower concentrations of probenecid in our study (300 μM *versus* 1 mM), or different species (mice *versus* rats) might be involved in these differences. Altogether, the present study does not exclude the possibility that probenecid may inhibit

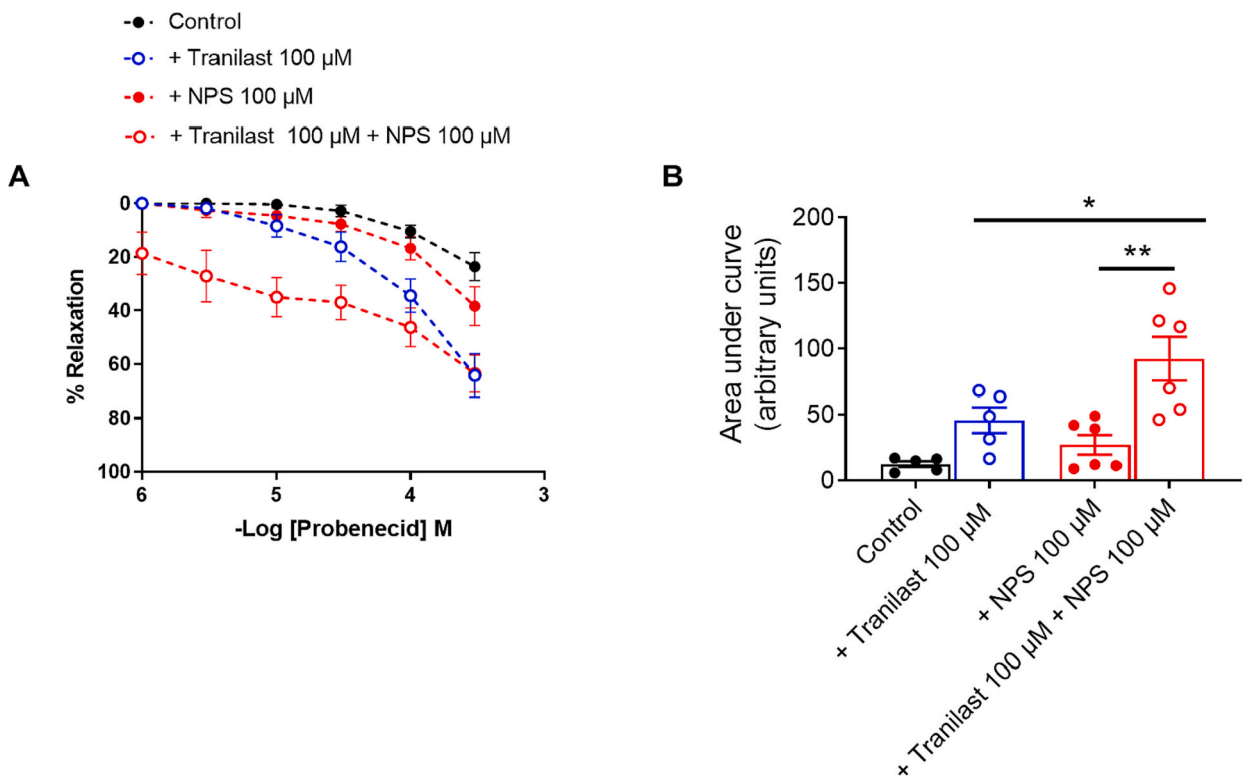

Fig. 8. Impact of NO exposure on the tranilast-induced potentiation of probenecid relaxations. (A) Impact of incubation with the NO donor, sodium nitroprusside (NPS, 100 μM), on the effect of the selective TRPV2 inhibitor, tranilast (100 μM), on the concentration-response curves to probenecid in U46619 pre-contracted abdominal aortas without endothelium. (B) Quantification of the area under curve of concentration-response curves to probenecid. Results are the mean ± SEM from 5 to 6 mice. *$P < 0.05$, **$P < 0.01$ by two-way ANOVA.

α-adrenergic receptors in the mouse aorta. However, α-adrenergic receptor signaling is not involved in mouse aorta probenecid relaxations in U46619 pre-contracted vessels, which we hypothesized could be dependent on TRPV2 channel signaling. To our knowledge, this is the first evidence of the possible involvement of TRPV2 channels in endothelial-dependent relaxations.

Different members of the TRPV channel family have been associated with endothelium-dependent relaxations. Thus, endothelial TRPV1 channels participate in NO release leading to rat mesenteric and pig coronary artery relaxations [36,37]. In addition, activation of TRPV4 channels induces NO- and EDHF-dependent vasodilation in rat and mouse conduit and resistance arteries [38–41]. A previous work revealed that TRPV2 channel activation evokes relaxation in mouse intestine through NO release from inhibitory motor neurons [30]. However, in the mouse aorta, inhibition of NO production by L-NAME did not significantly affect probenecid relaxations, which were only attenuated after either K_{ir} or K_v channel blockade. These results partly agree with those obtained in different TRPV2 channel-expressing cell lines in which K_v channel blockade led to TRPV2 channel inhibition [22]. The blockade of K_{ir} or K_v channels did not have an effect on endothelium-denuded vessels, which suggests the essential role of this vascular layer. At this stage, the connection between K_{ir} and K_v channels and endothelium-dependent relaxations to probenecid is not clear, but we propose that endothelial TRPV2 channel activation may release a mediator that would diffuse into the smooth muscle layer to activate K_v and K_{ir} channels (Fig. 9). Nevertheless, further studies are needed to corroborate this hypothesis.

The antiallergic drug tranilast has been used as a TRPV2 channel antagonist [28–30], because it is one of the most specific, commercially available TRPV2 channel inhibitors. Likewise, in the present study, we used tranilast to corroborate the role of TRPV2 channel activation on probenecid-induced vascular responses. Paradoxically, incubation with tranilast did not decrease but largely increased probenecid relaxations regardless of the presence of endothelium, suggesting that muscular TRPV2 channel activity exerts negative feedback on probenecid relaxations. In a previous study, it was shown that tranilast increases ACh-induced endothelium-dependent relaxations in rat mesenteric arteries, but not in the rat aorta [42]. Likewise, tranilast was not able to modify ACh relaxations in the mouse aorta, which excludes the participation of TRPV2 channels in these responses and, in turn, confirms the specific impact of TRPV2 channel inhibition on probenecid-induced relaxations. Notably, although the enhancement of relaxations after TRPV2 channel inhibition may be a consequence of the blockade of TRPV2 channel-induced smooth muscle Ca^{2+} entry leading to a decrease in smooth muscle tone, we propose that these findings might also reveal the presence of a direct effect of probenecid on an endothelium-independent relaxing mechanism, which would be counteracted by activation of TRPV2 channels located in smooth muscle cells.

In endothelium-denuded aortic rings, the tranilast-induced blockade of TRPV2 channels and the subsequent increase in probenecid relaxations was abrogated by K_{ATP} channel blockade, which suggests that the increased probenecid relaxant activity after TRPV2 channel inhibition could be related to these channels. Nonetheless, at this stage, neither the present work nor previous evidence completely supports that probenecid may directly activate K_{ATP} channels (Fig. 9). K_{ATP} channels couple cellular metabolism with membrane excitability, Ca^{2+} entry, and cell functions, and K_{ATP} channel blockade results in vasoconstriction and depolarization of vascular smooth muscle cells [43]. In the present study, antagonism of muscular TRPV2 channels with tranilast significantly decreased intracellular ATP concentrations in the mouse aorta, an environment that permits activation of K_{ATP} channels leading to smooth muscle hyperpolarization and relaxation. Besides, probenecid is reported to block pannexin-1 channels, which are master regulators of intracellular ATP concentrations [27,44]. However, in our study, the

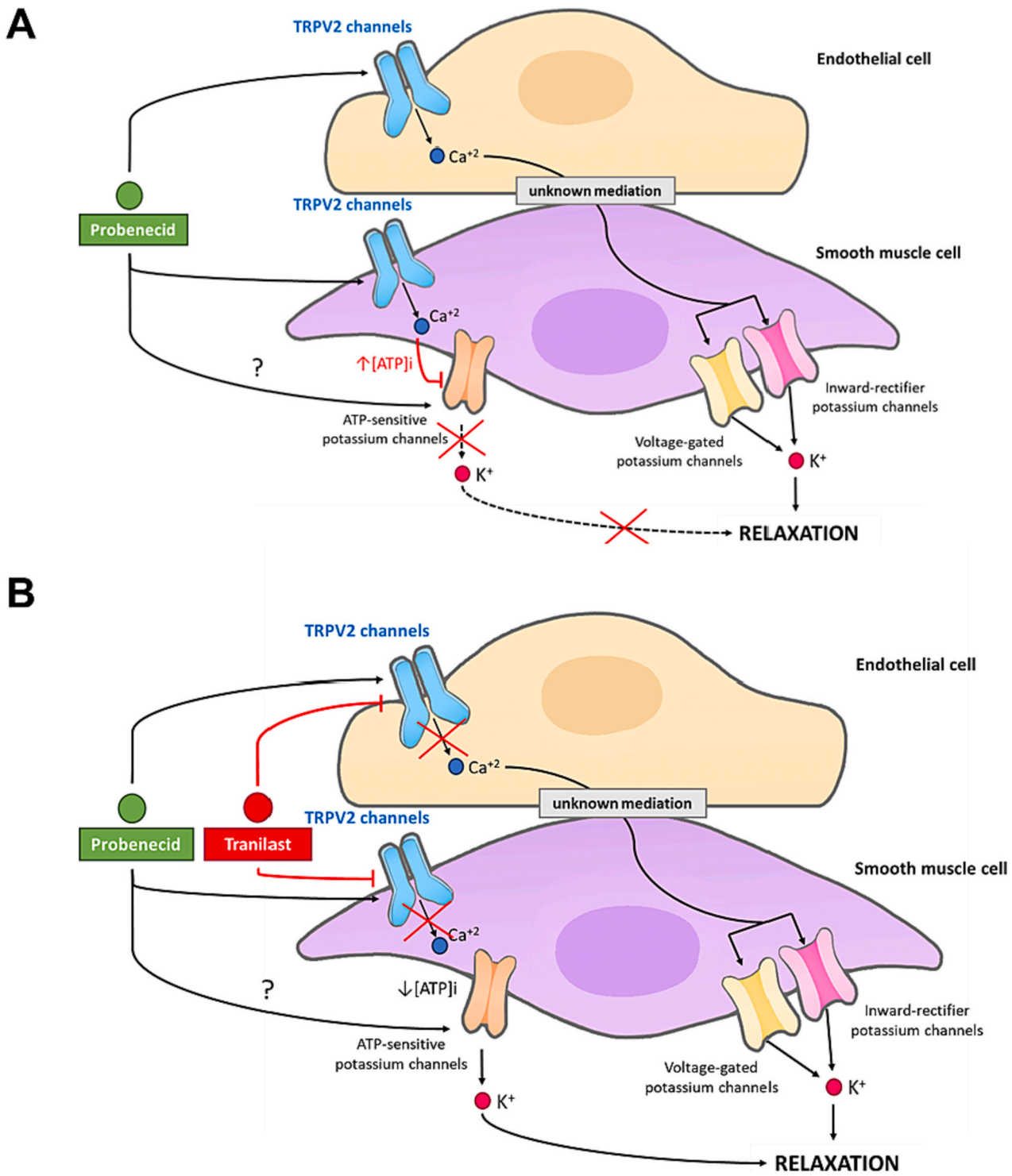

Fig. 9. Proposed mechanism of modulation of aortic tone by TRPV2 channels. (A) Probenecid by binding to TRPV2 channels located in endothelial cells induces the release of an unknown mediator, which activates voltage-gated and inwardly rectifying K^+ channels in smooth muscle cells, leading to membrane hyperpolarization and subsequent aortic relaxation. On the other hand, probenecid binding to smooth muscle TRPV2 channels increases intracellular ATP concentrations, which inhibit K_{ATP} channel activation inducing negative feedback on probenecid relaxations. (B) Tranilast-induced blockade of TRPV2 channels decreases intracellular ATP concentrations, which activates K_{ATP} channels, leading to increased endothelium-independent relaxations. A potential direct effect of probenecid on activating K_{ATP} channels could not be discarded.

pannexin-1 channel inhibitor carbenoxolone did not affect intracellular ATP concentrations in the presence of tranilast, suggesting that another mechanism or mechanisms may be involved in this effect. Taken together, we suggest that smooth muscle K_{ATP} channel opening is silenced by smooth muscle TRPV2 channel activation, though further studies are needed to define the precise mechanisms underlying the contribution of ATP in this novel signaling pathway.

Prior studies have shown that in response to NO exposure, TRPV2 channels located in the endoplasmic reticulum membrane translocate to the plasma membrane in a phosphoinositide 3-kinase-dependent

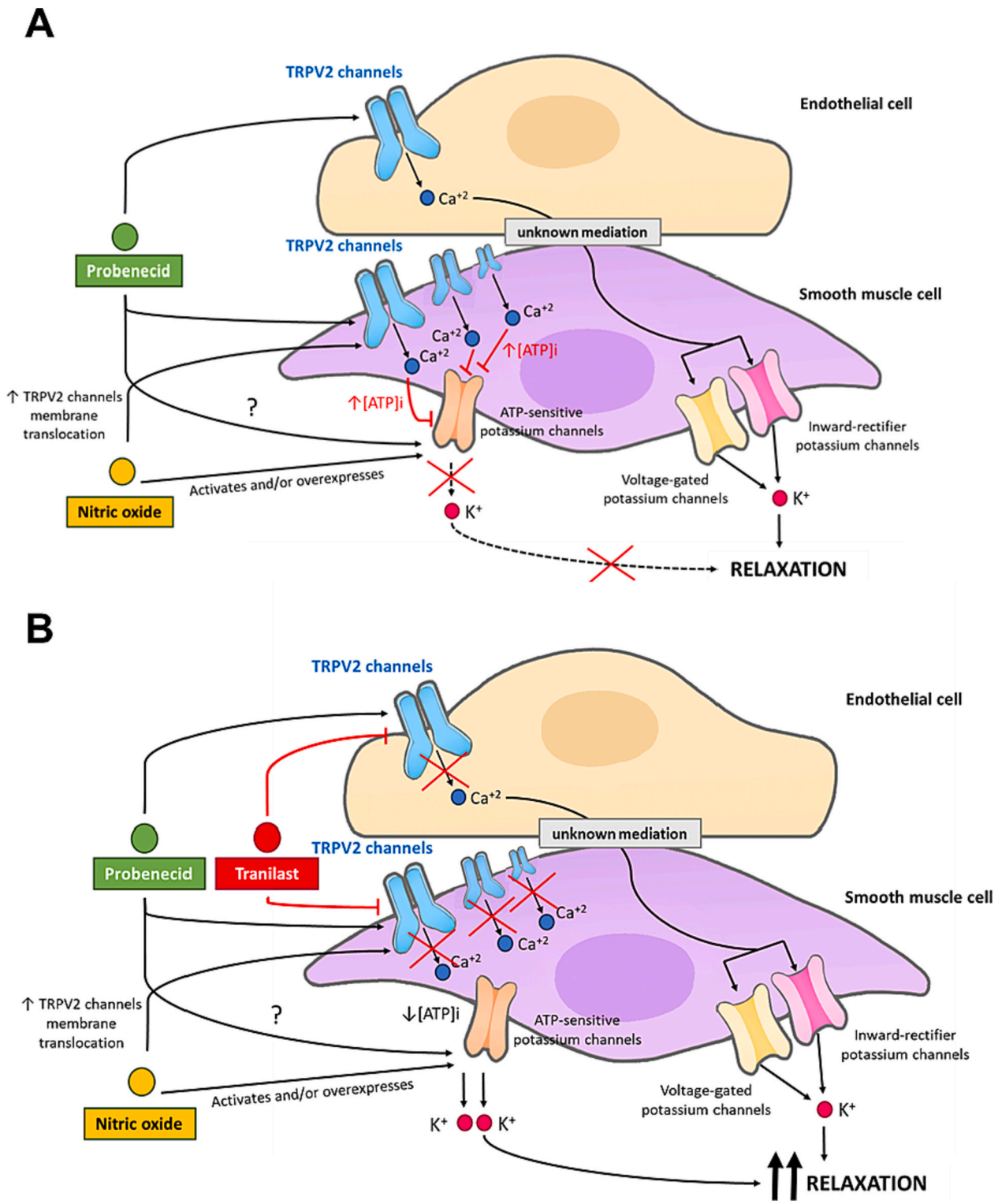

Fig. 10. Influence of nitric oxide on the proposed mechanism of modulation of aortic tone by TRPV2 channels. (A) Nitric oxide augments TRPV2 channel translocation to the plasma membrane and may also directly activate and/or overexpress K_{ATP} channels leading to increased channel activity, an effect that counteracts each other. (B) In the presence of tranilast, the blockade of K_{ATP} channels by TRPV2 channels is removed and this effect, in conjunction with a potential direct effect of nitric oxide on K_{ATP} channel activation and/or overexpression, jointly contributes to the enhancement of probenecid relaxations.

manner [28]. To find out whether NO increases plasma membrane translocation of TRPV2 channels in vascular cells, we exposed cultured aortic smooth muscle cells to the NO donor NPS. Incubation with this compound led to augmented TRPV2 channel translocation to the plasma membrane. This effect was associated with further potentiation of probenecid relaxations induced by tranilast and, thus, of the negative feedback exerted by TRPV2 channels, which was dependent on K_{ATP} channel activation. In fact, K_{ATP} channels are activated and overexpressed by NO in the rat aorta during septic shock [45], and additional evidence showed that NO activates these channels in rabbit

mesenteric artery vascular smooth muscle cells [46]. However, this activity was not observed in other vessels, such as rabbit mesenteric arterial [47] and pig coronary arterial [48] smooth muscle cells. Overall, these results argue in favor of a direct activation of K_{ATP} channels by probenecid but as mentioned above, this should be confirmed in subsequent studies (Fig. 10). In addition, these results suggest that NO is a modulator of smooth muscle cell TRPV2 channel function, which highlights the pathophysiological relevance of vascular TRPV2 signaling identified in the present study. A limitation of the present study is that we did not evaluate whether NO increases plasma membrane translocation of TRPV2 channels in endothelial cells and the potential functional impact on aortic relaxations. Future studies should investigate this potential modulation as a priority.

The pathophysiological importance of the relationship between NO, TRPV2 channels, and K_{ATP} channels could be better interpreted considering that increased iNOS-derived NO contributes to the pathogenesis of multiple vascular disorders, including hypertension [49]. This is mostly ascribed to nitrosative stress due to excessive NO production by iNOS, which after reaction with superoxide anion forms peroxynitrite. This harmful molecule subsequently leads to significantly increased oxidative/nitrosative stress and cellular damage in a vicious cycle. According to our hypothesis, a surge in iNOS-derived NO may increase the contribution of TRPV2 channels to vascular contractile responses by its translocation to the membrane, but may also induce a potential direct activation of K_{ATP} channels by NO. Given that reactive oxygen species have been reported to activate TRPV2 channel function and expression [50,51], we speculate that iNOS-derived NO may increase the negative feedback of TRPV2 channels on relaxations, which may constitute a novel mechanism involved in vasodilatory dysfunction that needs further attention.

5. Conclusion

Understanding the role of TRPV2 channels in the vasculature is crucial to comprehend vascular physiology and the potential mechanisms underlying vascular disease. The main limitation of this study is that the pharmacological tools used are non-selective TRPV2 channel-modulating drugs, indicating that the present results should be interpreted with caution. More potent and specific TRPV2 channel modulators are needed to confirm the findings of the present study. Nonetheless, here we provide the first evidence that TRPV2 channel activation may modulate mouse aortic tone by evoking endothelium-dependent relaxations and parallel negative feedback on smooth muscle K_{ATP} channel activation, leading to net vasodilation. Given that NO can enhance TRPV2 channel-induced smooth muscle negative feedback on relaxations, we suggest that this signaling pathway may be hyperactivated in conditions where NO availability is increased. Further research is needed to understand the impact of this abnormal regulation of vascular TRPV2 channel signaling mediated by NO, as this information may be crucial to develop new strategies for the prevention and treatment of vascular dysfunction.

Ethics statement

All procedures were carried out following the Spanish Legislation (RD35/2013) and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication number 85-23, revised in 1996). Likewise, the procedure was approved by the Ethics Committee of the Autonomous University of Barcelona (approval code: FJA-eut/01).

CRediT authorship contribution statement

AP-M and FJ-A conceptualized and designed the experiments. MS, JS, AT, BP, CP, and FJ-A participated on data acquisition, analysis, and visualization. FJ-A supervised the study and wrote the first draft of the paper. AP-M, MS, JS, AT, BP, CP, GM, MJ, PD'O and FJ-A participated in data interpretation and manuscript drafting and editing. All authors approved the final version of the manuscript; agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; and qualify for authorship, while all those who qualify for authorship are listed.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be interpreted as a potential conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2023.122286.

References

[1] A. Samanta, Y. Liu, T.E.T. Hughes, S. Zhao, Y. Yudin, T. Rohacs, S. Han, V. Y. Moiseenkova-Bell, Molecular mechanism of TRPV2 channel modulation by cannabidiol, eLife 8 (2019) 1–17.
[2] M.J. Caterina, Z. Pang, TRP channels in skin biology and pathophysiology, Pharmaceuticals 9 (4) (2016) 77.
[3] M.G. Rosasco, S.E. Gordon, TRP channels: what do they look like, in: Neurobiology of TRP Channels, 2017, pp. 1–9.
[4] A. Haustrate, N. Prevarskaya, Lehen'kyi V., Role of the TRPV channels in the endoplasmic reticulum calcium homeostasis, Cells 9 (2) (2020) 1–8.
[5] A. Perálvarez-Marín, P. Doñate-Macian, R. Gaudet, What do we know about the transient receptor potential vanilloid 2 (TRPV2) ion channel? FEBS J. 280 (21) (2013) 5471–5487.
[6] S.F. Pedersen, G. Owsianik, B. Nilius, TRP channels: an overview, Cell Calcium 38 (3–4) (2005) 233–252.
[7] B. O'Connor, N. Robbins, S.E. Koch, J. Rubinstein, TRPV2 channel-based therapies in the cardiovascular field. Molecular underpinnings of clinically relevant therapies, Prog. Biophys. Mol. Biol. 159 (2021) 118–125.
[8] K. Shibasaki, Y. Ishizaki, S. Mandadi, Astrocytes express functional TRPV2 ion channels, Biochem. Biophys. Res. Commun. 441 (2013) 327–332.
[9] X.R. Yang, M.J. Lin, L.S. McIntosh, J.S.K. Sham, Functional expression of transient receptor potential melastatin- and vanilloid-related channels in pulmonary arterial and aortic smooth muscle, Am. J. Physiol. Lung Cell. Mol. Physiol. 290 (6) (2006) L1267–L1276.
[10] K.S. Park, Y. Kim, Y.H. Lee, Y.E. Earm, W.K. Ho, Mechanosensitive cation channels in arterial smooth muscle cells are activated by diacylglycerol and inhibited by phospholipase C inhibitor, Circ. Res. 93 (2003) 557–564.
[11] K. Muraki, Y. Iwata, Y. Katanosaka, T. Ito, S. Ohya, M. Shigekawa, Y. Imaizumi, TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes, Circ. Res. 93 (9) (2003) 829–838.
[12] I. Fantozzi, S. Zhang, O. Platoshyn, C.V. Remillard, R.T. Cowling, J.X. Yuan, Hypoxia increases AP-1 binding activity by enhancing capacitative Ca2+ entry in human pulmonary artery endothelial cells, Am. J. Physiol. Lung Cell. Mol. Physiol. 285 (2003) L1233–L1245.
[13] Y. Iwata, Y. Katanosaka, Y. Arai, K. Komamura, K. Miyatake, M. Shigekawa, A novel mechanism of myocyte degeneration involving the Ca2+-permeable growth factor-regulated channel, J. Cell Biol. 161 (2003) 957–967.
[14] Z. Yue, J. Xie, A.S. Yu, J. Stock, J. Du, L. Yue, Role of TRP channels in the cardiovascular system, Am. J. Physiol. Heart Circ. Physiol. 308 (2015) H157–H182.

[15] Y. Iwata, S. Ito, S. Wakabayashi, M. Kitakaze, TRPV2 channel as a possible drug target for the treatment of heart failure, Lab. Investig. 100 (2) (2020) 207–217.
[16] M. Entin-Meer, G. Keren, Potential roles in cardiac physiology and pathology of the cation channel TRPV2 expressed in cardiac cells and cardiac macrophages: a mini-review, Am. J. Physiol. Heart Circ. Physiol. 318 (1) (2020) H181–H188.
[17] C. Lorin, I. Vögeli, E. Niggli, Dystrophic cardiomyopathy: role of TRPV2 channels in stretch-induced cell damage, Cardiovasc. Res. 106 (2015) 153–162.
[18] M. Entin-Meer, L. Cohen, E. Hertzberg-Bigelman, R. Levy, J. Ben-Shoshan, G. Keren, TRPV2 knockout mice demonstrate an improved cardiac performance following myocardial infarction due to attenuated activity of peri-infarct macrophages, PLoS One 12 (2017), e0177132.
[19] J.A. Fernández, D.P. Dash, J. McKee, D.A. Simpson, A.V. Zholos, J.G. McGeown, T. M. Curtis, TRPV2 channels contribute to stretch-activated cation currents and myogenic constriction in retinal arterioles, Invest. Ophthalmol. Vis. Sci. 57 (13) (2016) 5637–5647.
[20] M.J.E. Maksoud, V. Tellios, D. An, Y.Y. Xiang, W.Y. Lu, Nitric oxide upregulates microglia phagocytosis and increases transient receptor potential vanilloid type 2 channel expression on the plasma membrane, Glia 67 (12) (2019) 2294–2311.
[21] H.Z. Hu, Q. Gu, C. Wang, C.K. Colton, J. Tang, M. Kinoshita-Kawada, L.Y. Lee, J. D. Wood, M.X. Zhu, 2-Aminoethoxydiphenyl borate is a common activator of TRPV1, TRPV2, and TRPV3, J. Biol. Chem. 279 (2004) 35741–35748.
[22] V. Juvin, A. Penna, J. Chemin, Y.L. Lin, F.A. Rassendren, Pharmacological characterization and molecular determinants of the activation of transient receptor potential V2 channel orthologs by 2-aminoethoxydiphenyl borate, Mol. Pharmacol. 72 (2007) 1258–1268.
[23] J.B. Park, S.J. Kim, Anti-hypertensive effects of probenecid via inhibition of the α-adrenergic receptor, Pharmacol. Rep. 63 (5) (2011) 1145–1150.
[24] S.P.H. Alexander, E. Kelly, A. Mathie, J.A. Peters, E.L. Veale, J.F. Armstrong, E. Faccenda, S.D. Harding, A.J. Pawson, C. Southan, O.P. Buneman, A. Christopoulos, J.A. Cidlowski, A.P. Davenport, D. Fabbro, M. Spedding, J. Striessnig, J.A. Davies, C.G.T.P. Collaborators, The concise guide to pharmacology 2021/22, Br. J. Pharmacol. 178 (Suppl. 1) (2021) S1–S513.
[25] S. Bang, K.Y. Kim, S. Yoo, S.-H. Lee, S.W. Hwang, Transient receptor potential V2 expressed in sensory neurons is activated by probenecid, Neurosci. Lett. 425 (2) (2007) 120–125.
[26] N. Robbins, S.E. Koch, M. Tranter, J. Rubinstein, The history and future of probenecid, Cardiovasc. Toxicol. 12 (2011) 1–9.
[27] Z. Onódi, S. Koch, J. Rubinstein, P. Ferdinandy, Z.V. Varga, Drug repurposing for cardiovascular diseases: new targets and indications for probenecid, Br. J. Pharmacol. 180 (6) (2023) 685–700.
[28] K. Aoyagi, M. Ohara Imaizumi, C. Nishiwaki, Y. Nakamichi, S. Nagamatsu, Insulin/phosphoinositide 3-kinase pathway accelerates the glucose-induced first-phase insulin secretion through TrpV2 recruitment in pancreatic b-cells, Biochem. J. 432 (2010) 375–386.
[29] E. Hisanaga, M. Nagasawa, K. Ueki, R.N. Kulkarni, M. Mori, I. Kojima, Regulation of calcium-permeable TRPV2 channel by insulin in pancreaticbeta-cells, Diabetes 58 (2009) 174–184.
[30] H. Mihara, A. Boudaka, K. Shibasaki, A. Yamanaka, T. Sugiyama, M. Tominaga, Involvement of TRPV2 activation in intestinal movement through nitric oxide production in mice, J. Neurosci. 30 (2010) 16536–16544.
[31] F. Jiménez-Altayó, A. Cabrera, A. Bagán, L. Giménez-Llort, P. D'Ocon, B. Pérez, M. Pallàs, C. Escolano, An imidazoline 2 receptor ligand relaxes mouse aorta via off-target mechanisms resistant to aging, Front. Pharmacol. 13 (2022), 826837.
[32] E. Vila, M. Solé, N. Masip, L. Puertas-Umbert, S. Amaro, A.P. Dantas, M. Unzeta, P. D'Ocon, A.M. Planas, Á. Chamorro, F. Jiménez-Altayó, Uric acid treatment after stroke modulates the Krüppel-like factor 2-VEGF-A axis to protect brain endothelial cell functions: impact of hypertension, Biochem. Pharmacol. 164 (2019) 115–128.
[33] M. Solé, M. Unzeta, Vascular cell lines expressing SSAO/VAP-1: a new experimental tool to study its involvement in vascular diseases, Biol. Cell. 103 (11) (2011) 543–557.
[34] J. Sudo, J. Terui, H. Iwase, K. Kakuno, Assay of ATPase and Na,K-ATPase activity using high-performance liquid chromatographic determination of ADP derived from ATP, J. Chromatogr. B Biomed. Sci. Appl. 744 (1) (2000) 19–23.
[35] M. Nyberg, P. Piil, O.T. Kiehn, C. Maagaard, T.S. Jørgensen, J. Egelund, et al., Probenecid inhibits α-adrenergic receptor-mediated vasoconstriction in the human leg vasculature, Hypertension 71 (1) (2018) 151–159.
[36] I.M. Poblete, M.L. Orliac, R. Briones, E. Adler-Graschinsky, J.P. Huidobro-Toro, Anandamide elicits an acute release of nitric oxide through endothelial TRPV1 receptor activation in the rat arterial mesenteric bed, J. Physiol. 568 (2005) 539–551.
[37] I.N. Bratz, G.M. Dick, J.D. Tune, J.M. Edwards, Z.P. Neeb, U.D. Dincer, M. Sturek, Impaired capsaicin-induced relaxation of coronary arteries in a porcine model of the metabolic syndrome, Am. J. Physiol. Heart Circ. Physiol. 294 (2008) H2489–H2496.
[38] R. Köhler, W.T. Heyken, P. Heinau, R. Schubert, H. Si, M. Kacik, C. Busch, I. Grgic, T. Maier, J. Hoyer, Evidence for a functional role of endothelial transient receptor potential V4 in shear stress-induced vasodilatation, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 1495–1502.
[39] S.P. Marrelli, R.G. O'Neil, R.C. Brown, R.M. Bryan Jr., PLA2 and TRPV4 channels regulate endothelial calcium in cerebral arteries, Am. J. Physiol. Heart Circ. Physiol. 292 (2007) H1390–H1397.
[40] D.X. Zhang, S.A. Mendoza, A.H. Bubolz, A. Mizuno, Z.D. Ge, R. Li, D.C. Warltier, M. Suzuki, D.D. Gutterman, Transient receptor potential vanilloid type 4-deficient mice exhibit impaired endothelium-dependent relaxation induced by acetylcholine in vitro and in vivo, Hypertension 53 (2009) 532–538.
[41] S.K. Sonkusare, A.D. Bonev, J. Ledoux, W. Liedtke, M.I. Kotlikoff, T.J. Heppner, D. C. Hill-Eubanks, M.T. Nelson, Elementary Ca2+ signals through endothelial TRPV4 channels regulate vascular function, Science 336 (2012) 597–601.
[42] F.E. Xavier, J. Blanco-Rivero, E. Sastre, L. Caracuel, M. Callejo, G. Balfagón, Tranilast increases vasodilator response to acetylcholine in rat mesenteric resistance arteries through increased EDHF participation, PLoS One 9 (7) (2014) 1–9.
[43] E.A. Ko, J. Han, I.D. Jung, W.S. Park, Physiological roles of K+ channels in vascular smooth muscle cells, J. Smooth Muscle Res. 44 (2) (2008) 65–81.
[44] W. Silverman, S. Locovei, G. Dahl, Probenecid, a gout remedy, inhibits pannexin 1 channels, Am. J. Physiol. Cell Physiol. 295 (3) (2008) C761–C767.
[45] S. Collin, N. Sennoun, A.G. Dron, M. de la Bourdonnaye, C. Montemont, P. Asfar, P. Lacolley, F. Meziani, B. Levy, Vascular ATP-sensitive potassium channels are over-expressed and partially regulated by nitric oxide in experimental septic shock, Intensive Care Med. 37 (5) (2011) 861–869.
[46] M.E. Murphy, J.E. Brayden, Nitric oxide hyperpolarizes mesenteric arteries via ATP-sensitive potassium channels, J. Physiol. 486 (Pt 1) (1995) 47–58.
[47] J.M. Quayle, A.D. Bonev, J.E. Brayden, M.T. Nelson, Calcitonin gene-related peptide activated ATP sensitive K+ currents in rabbit arterial smooth muscle via protein kinase A, J. Physiol. 475 (1) (1994) 9–13.
[48] G.C. Wellman, J.M. Quayle, N.B. Standen, ATP-sensitive K+ channel activation by calcitonin gene related peptide and protein kinase A in pig coronary arterial smooth muscle, J. Physiol. 507 (Pt1) (1998) 117–129.
[49] H.N. Siti, Y. Kamisah, J. Kamsiah, The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review), Vasc. Pharmacol. 71 (2015) 40–56.
[50] T.C. Fricke, F. Echtermeyer, J. Zielke, J. de la Roche, M.R. Filipovic, S. Claverol, C. Herzog, M. Tominaga, R.A. Pumroy, V.Y. Moiseenkova-Bell, P.M. Zygmunt, A. Leffler, M.J. Eberhardt, Oxidation of methionine residues activates the high-threshold heat-sensitive ion channel TRPV2, Proc. Natl. Acad. Sci. U. S. A. 116 (48) (2019) 24359–24365.
[51] J. Enrich-Bengoa, G. Manich, T. Valente, P. Sanchez-Molina, B. Almolda, C. Solà, J. Saura, B. González, B. Castellano, A. Perálvarez-Marín, TRPV2: a key player in myelination disorders of the central nervous system, Int. J. Mol. Sci. 23 (7) (2022) 3617.