TRPC3 mediates pyrimidine receptor-induced depolarization of cerebral arteries

S. A. Reading, S. Earley, B. J. Waldron, D. G. Welsh, and J. E. Brayden
Department of Pharmacology, University of Vermont, Burlington, Vermont

Submitted 23 August 2004; accepted in final form 13 December 2004

TRPC3 mediates pyrimidine receptor-induced depolarization of cerebral arteries. Am J Physiol Heart Circ Physiol 288: H2055–H2061, 2005. First published December 16, 2004; doi:10.1152/ajpheart.00861.2004.—We tested the hypothesis that TRPC3, a member of the canonical transient receptor potential (TRP) family of channels, mediates agonist-induced depolarization of arterial smooth muscle cells (SMCs). In support of this hypothesis, we observed that suppression of arterial SMC TRPC3 expression with antisense oligodeoxynucleotides significantly decreased the depolarization and constriction of intact cerebral arteries in response to UTP. In contrast, depolarization and contraction of SMCs induced by increased intravascular pressure, i.e., myogenic responses, were not altered by TRPC3 suppression. Interestingly, UTP-evoked responses were not affected by suppression of a related TRP channel, TRPC6, which was previously found to be involved in myogenic depolarization and vasoconstriction. In patch-clamp experiments, UTP activated a whole cell current that was greatly reduced or absent in TRPC3 antisense-treated SMCs. These results indicate that TRPC3 mediates UTP-induced depolarization of arterial SMCs and that TRPC3 and TRPC6 may be differentially regulated by receptor activation and mechanical stimulation, respectively.

transient receptor potential; nonselective cation channels; vascular smooth muscle; uridine triphosphate; vasoconstriction; antisense oligodeoxynucleotides

ARTERIAL DIAMETER is a primary effector of blood flow and pressure. Influx of extracellular Ca2+ through voltage-dependent L-type Ca2+ channels located in the arterial smooth muscle cell (SMC) plasma membrane is central in the control of cerebrovascular arterial diameter (24). Membrane depolarization opens L-type Ca2+ channels, and their steep voltage dependence means that small changes in membrane potential (V_m) dramatically affect channel open probability, Ca2+ influx, and vascular tone. Various agonists that bind to receptors on the SMC plasma membrane and activate the phospholipase C-inositol 1,4,5-trisphosphate-diacylglycerol signal transduction pathway [norepinephrine (8, 23, 25), histamine (3, 7), 5-hydroxytryptamine (23), and UTP (39)] are known to depolarize and constrict arterial SMCs. An unresolved issue in vascular biology is the identification and characterization of the membrane channels responsible for agonist-induced depolarization of arterial smooth muscle.

The recent identification of mammalian homologs of the Drosophila transient receptor potential (TRP) channel in native vascular SMCs raised the interesting possibility that mammalian TRPCs mediate agonist-induced membrane depolarization. Members of the canonical TRP channel family (TRPC), specifically TRPC3 and TRPC6, are found in rat aorta (5), preglomerular resistance vessels (5), rabbit and mouse portal veins (12), and rat cerebral artery (40). TRPC3 and TRPC6 channels are activated by diacylglycerol independent of PKC (37) and give rise to a cation current that has relatively low selectivity for Ca2+ over Na+ (10). Recently, TRPC6 channels were reported to mediate a nonselective cation current activated by α1-adrenergic receptor stimulation in rabbit portal vein SMCs (12) and in rat embryonic aorta SMCs exposed to vasopressin (14). A clear role for TRPC6 regulation of myogenic tone in rat cerebrovascular resistance arteries has also been demonstrated (40). However, in contrast to TRPC6, a role for TRPC3 channels in native vascular SMCs has not been established.

The focus of the present study was to determine whether TRPC3 or TRPC6 channels are involved in agonist-induced depolarization of cerebral artery SMCs. We observed that antisense oligodeoxynucleotide (ODN) suppression of TRPC3, but not TRPC6, expression attenuated UTP-induced depolarization and constriction of cerebral arteries and abolished a UTP-activated ion current in isolated arterial SMCs. These results demonstrate that TRPC3 is specifically involved in agonist-evoked arterial SMC constriction.

MATERIALS AND METHODS

Animals and tissues. Twelve- to 16-wk-old male Sprague-Dawley rats (Charles River Laboratories, St. Constant, PQ, Canada) were studied. All animal use procedures were in accordance with institutional guidelines and approved by the institutional Animal Care and Use Committee at the University of Vermont.

Rats were euthanized with an injection of pentobarbitone sodium (150 mg/kg ip) followed by exsanguination. The brain was removed, and cerebellar and cerebral arteries (125–225 μm diameter) were dissected free in ice-cold MOPS-buffered saline solution containing 3 mM MOPS, 145 mM NaCl, 5 mM KCl, 1 mM MgSO4·7H2O, 2.5 mM CaCl2, 1 mM KH2PO4, 0.02 mM EGTA, 2 mM pyruvate, 5 mM glucose, and 1% bovine serum albumin (pH 7.4).

RT-PCR analysis. RNA was prepared from arteries or isolated SMCs with use of the RNeasy kit (Qiagen, Valencia, CA). Then 3–5 μl of each first-strand cDNA reaction was placed in 40–45 μl of a PCR solution (Applied Biosystems, Branchburg, NJ) containing 1.4 mM MgCl2, 20 μM forward and reverse primers (Great American Gene, Ramona, CA), 0.25 mM deoxyxynucleotide triphosphates, 1× reaction buffer, and 2.5 U of AmpliTaq Gold DNA polymerase. PCR were hot started (94°C for 10 min) and then exposed to 35–40 cycles of 94°C for 60 s, 50°C for 90 s, and 72°C for 60 s. Forward and reverse primers specific for TRPC3 were designed using Vecti NTI software: 5′-CCTGAGCGAAGTCACACTCCCAC-3′ (forward) and 5′-CCTGAGCGAAGTCACACTCCCAC-3′ (reverse). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
and 5'-CCACTCTACATCAGTGTACC-3' (reverse). Primers yield product sizes of 529 bp for TRPC3. All reaction products were resolved on 1% agarose gels.

**Western analysis.** Arterial segments were homogenized in lysis buffer (5 min at 4°C) containing 40 mM 3-(Cyclohexylamino)-1-propanesulfonic acid, 1 mM dl-dithiothreitol, 10 mM EDTA, 15 mM MgCl₂, 115 mM NaCl, 1 mM Na-orthovanadate, 1 mM NaF, 2.5 mM urca, 0.25% deoxycholate, 10% glycerol, 1% NP-40, 0.2% SDS, and 1:500 mammalian protease inhibitor cocktail (Sigma, St. Louis, MO). Equal amounts of sample protein were separated on a 10% polyacrylamide gel and transferred to a nitrocellulose membrane. The membranes were exposed to a TRPC3 or TRPC6 polyclonal antibody (anti-rabbit, 1:200 dilution; Alomone Labs, Jerusalem, Israel) and a glyceraldehyde dehydrogenase (GAPDH) monoclonal antibody (antimouse, 1:1,000 dilution; Chemicon Labs, Temecula, CA). Alexa Fluor 680 goat anti-rabbit (Molecular Probes, Eugene, OR) and IRDye 800 anti-mouse (Rockland Immunochemicals, Gilbertsville, PA) were used to fluorescently label the TRPC3 and GAPDH antibodies, respectively. The density of signals specific for the TRPC3 and GAPDH bands in a given lane on a membrane was measured after the membrane was scanned with an Odyssey infrared imaging system (Li-COR Biosciences, Lincoln, NE).

**Immunohistochemistry.** Freshly isolated arterial segments were fixed for 15 min in phosphate-buffered saline containing 4% paraformaldehyde and exposed to the primary antibody (1:250 dilution of rabbit anti-TRPC3; Alomone Labs) overnight. Alexa Fluor 680 goat anti-rabbit (Molecular Probes, Eugene, OR) was used to fluorescently label the TRPC3 antibody. The arteries were examined at ×40 magnification using a laser scanning confocal microscope (model 1000, Bio-Rad).

**ODN sequences and reverse permeabilization.** TRPC6 sense and antisense ODNs were designed as previously described (40): 5'-CCCTAGCCAGTCTGAACTCC-3' (antisense) and 5'-GAGAACCATGAACTGGAATA-3' (sense). The last five bases on the 3' end were phosphorothioated to limit ODN degradation. For some experiments, fluorescein isothiocyanate was conjugated to the 5' end to allow for histological assessment of cellular uptake of the ODNs. All ODNs were synthesized and HPLC purified commercially by Qiagen (Alameda, CA).

Sense and antisense ODNs (2 μM) were introduced into the arterial SMCs via a reversible-permeabilization procedure (16). The arterial segments were then organ cultured for 3 days in DMEM-F-12 with 10 glucose. The pipette solution contained 120 mM CsCl, 3 mM MgCl₂, 0.1 mM EGTA, 10 mM HEPES (pH 7.2), 10 mM glucose, and 200 μM/ml amphotericin B. A series resistance of ~40 MΩ was accepted for all perforated-patch-clamp experiments. Membrane currents were filtered at 1 kHz, digitized at 5 kHz, and stored in a personal computer system for subsequent analysis. pClamp 8.1 and Clampfit 8.1 (Axon Instruments) were used to record and analyze membrane currents. Cell capacitance was measured with the cancelation circuitry of the voltage-clamp amplifier (Axopatch 200A, Axon Instruments). All current recordings were performed at room temperature (22°C).

**Diameter and membrane potential recordings.** Endothelium cell-denuded artery segments were mounted on glass pipettes in an arteriograph chamber (Living Systems, Burlington, VT), pressurized to 20 mmHg (with no flow), and superfused with warm (37°C), gassed (95% O₂-5% CO₂) physiological saline solution containing (in mM) 119 NaCl, 4.7 KCl, 24 NaHCO₃, 0.2 KH₂PO₄, 1.1 EDTA, 1.2 MgSO₄, 1.6 CaCl₂, and 10.6 glucose (pH 7.4). In experiments using Ca²⁺-free physiological saline solution, CaCl₂ was omitted and 3 mM EDTA and 30 μM diltiazem were added. The endothelium was removed as previously described (33). For verification of endothelial cell removal, the arteries were pressurized to 60 mmHg and allowed to develop myogenic tone before the vessels were exposed to 1 μM UTP. Absence of a dilation or biphasic constrictor response to 1 μM UTP indicated successful endothelial cell removal (20, 21).

Arterial diameter or Vₒ of sense- and antisense-treated arteries was measured in the absence (control) or presence of UTP (0.1–10 μM). Vₒ was measured by insertion of a sharp glass electrode (~100 MΩ resistance) containing 0.5 M KCl into the vessel wall. The criteria for successful vascular SMC impalement were 1) a sharp negative Vₒ deflection on entry, 2) a stable potential for ≥1 min after entry, and 3) a sharp positive Vₒ deflection on removal. Measurements were made using an electrometer (World Precision Instruments), and the data were recorded via computer using Axotape and Datanet software.

Arterial diameter was measured using a video dimension analyzer (IonOptix, Milton, MA).

**Chemicals, drugs, and enzymes.** Buffer reagents, collagenase type II, hyaluronidase, dithioerythritol, and UTP were purchased from Sigma. Papain was obtained from Worthington Biochemical (Lake-wood, NJ). Nisoldipine (a gift from Miles Pharmaceuticals, West Haven, CT) was dissolved in ethyl alcohol to a final solvent concentration of 0.1%. All other compounds were dissolved in the appropriate salt solution.

**Statistical analysis.** Values are means ± SE, and n indicates the number of animals. Changes in arterial diameter were measured as percent constriction, calculated as follows:

\[
\text{% constriction} = \frac{\text{initial diameter} - \text{diameter after UTP exposure}}{\text{initial diameter}} \times 100
\]

Student’s t-test was used to compare sense- with antisense-treated experimental groups. In experiments where the treatment group was exposed to more than one concentration of UTP, a two-way repeated-measures ANOVA was used. Means were considered significantly different at P ≤ 0.05.

**RESULTS**

Expression of TRPC3 in rat cerebral arteries. RT-PCR was used to determine whether mammalian TRPC3 mRNA transcripts were expressed in cerebral arteries of adult male rats. Message for TRPC3 was identified in intact cerebral arteries as well as in SMCs isolated from these arteries (Fig. 1A). Western analysis of arterial homogenates detected a protein band of ~120 kDa that was not detected when the TRPC3 antibody was preabsorbed with the peptide antigen (Fig. 1B). Immuno-
fluorescent labeling of intact cerebral arteries revealed a circumferential staining pattern for TRPC3 consistent with localization of TRPC3 to the arterial smooth muscle (Fig. 1C).

**Suppression of TRPC3 expression in cerebral artery.** Specific pharmacological inhibitors are not available to test the functional role of TRPC3 channels in vascular smooth muscle. Therefore, we employed an antisense ODN approach that we successfully used in previous studies to reduce TRPC6 channel expression and function (40). In the present study, we found that TRPC3 antisense ODNs decreased the expression of TRPC3 compared with sense-treated arteries. Fluorescein-labeled ODNs are taken up by cerebral arterial SMCs, and the uptake is significantly enhanced by reversibly permeabilizing the arteries (Fig. 2A). Western analysis showed that antisense treatment had no effect on the expression of GAPDH but reduced the density of the TRPC3 protein band after 3 days of organ culture (Fig. 2B); the TRPC3-to-GAPDH ratio was $42.5 \pm 11.4\%$ less in antisense-treated ($n=4$) than in sense-treated ($n=4$) samples (Fig. 2C). On the basis of previous studies (22, 34, 40), changes in protein expression of this magnitude are likely to be associated with altered activity of signaling systems that involve the protein of interest. TRPC3 antisense ODNs had no effect on the expression of TRPC6 in cerebral arterial SMCs (Fig. 2, B and C).

**Evidence of a functional role for TRPC3 in rat cerebral arteries.** We observed that UTP-induced depolarization of SMCs was significantly less in antisense- than in sense-treated arteries (Fig. 3A) at all UTP concentrations tested. In addition to attenuating UTP-induced depolarization of arterial SMCs, suppression of TRPC3 expression also reduced the constrictor responses to UTP over that same concentration range (Fig. 3B). Compared with sense-treated arteries, UTP-induced constrictions of TRPC3 antisense-treated arteries were reduced by $61\%$ in response to $10^{-6}$ M UTP and by $37\%$ in response to...

![Fig. 1. Transient receptor potential channel (TRPC3) is expressed in rat cerebral artery vascular smooth muscle. A: TRPC3 mRNA was detected in cerebral artery and vascular smooth muscle cells (SMCs) using RT-PCR. PCR was run with (+) and without (−) reverse transcriptase to verify the absence of genomic DNA contamination. B: Western blots of cerebral artery samples exposed to TRPC3 antibody (Ab) in the presence and absence of TRPC3 antigenic peptide. The band with a molecular mass (MW) of $\sim 120$ kDa was not apparent in the presence of the antigenic peptide. C: immunofluorescent staining of cerebral artery. Left: positive staining for anti-TRPC3 antibody in red. Right: lack of positive staining for anti-TRPC3 antibody in the presence of the antigen peptide. Dashed white line defines outer diameter of the arterial segment.](http://ajpheart.physiology.org/)

![Fig. 2. Reversible permeabilization (R-P) enhances oligodeoxynucleotide (ODN) uptake, and ODNs suppress TRPC3 expression in rat cerebral arteries. A: entry of fluorescein-labeled ODNs is greatly enhanced by reversible permeabilization compared with arteries exposed to ODNs in PBS for an equivalent amount of time. B: Western blot showing effect of TRPC3 antisense ODNs on TRPC3 and TRPC6 (red) and GAPDH (green) protein expression in cerebral artery. C: summary data for the effect of TRPC3 sense and antisense ODNs on TRPC3 and TRPC6 protein expression in rat cerebral artery. TRPC-to-GAPDH band density ratio was determined for each sense and antisense sample and then multiplied by 100 to yield a whole number. Values are means ± SE. *Significant difference ($P \leq 0.05$) between sense ($n=4$) and antisense ($n=4$) samples.](http://ajpheart.physiology.org/)
TRPC3 antisense ODNs inhibit a UTP-activated whole cell current. In further support of the involvement of TRPC3 in the depolarization and constriction induced by UTP, we have found that 30 μM UTP activates a whole cell current in SMCs isolated from TRPC3 sense-treated arteries (8 of 9 cells, voltage ramps from −120 to 20 mV; Fig. 5A). This response to UTP was absent in three of five SMCs and was greatly suppressed in two of five SMCs isolated from TRPC3 antisense-treated arteries (Fig. 5, B and C). These results demonstrate the presence of a UTP-activated current in cerebrovascular SMCs and strongly suggest that this current is mediated by TRPC3 channels.

Further evidence of a role for TRPC3 in agonist-evoked depolarization. In a final series of experiments, TRPC3 sense- and antisense-treated arteries were exposed to UTP concentrations sufficient to constrict the arteries by ~40%. In the continued presence of UTP, sense- and antisense-treated arteries were exposed to 10−6 M nisoldipine to inhibit voltage-dependent L-type Ca2+ channels. Consistent with the proposal that agonist-induced membrane depolarization contributes to vasoconstriction, we observed that blockade of the L-type Ca2+ channels with nisoldipine partially reversed the contractile response to UTP in sense-treated (Fig. 6), but not antisense-treated, arteries. These results strongly suggest that TRPC3 channels mediate UTP-induced depolarization of arterial SMCs and that the depolarization accounts for a substantial component of the overall vasoconstrictor response.

10−5 M UTP. Antisense ODNs had no generalized inhibitory effect on arterial contractility. Elevation of extracellular KCl from 5 to 60 mM decreased the resting diameter of sense- and antisense-treated arteries by 58 ± 12% (n = 6) and 57 ± 9% (n = 7), respectively. Similarly, pressure-induced depolarization and myogenic tone were identical in TRPC3 sense- and antisense-treated arteries. The arterial SMCs depolarized by 14 ± 1 mV in TRPC3 sense-treated (n = 6) and antisense-treated (n = 7) arteries and developed 26 ± 4% (TRPC3 sense) and 27 ± 5% (TRPC3 antisense) myogenic tone when intravascular pressure was increased from 20 to 80 mmHg.

Agonist-induced depolarization is not mediated by TRPC6 in cerebral artery. We previously showed that TRPC6 is involved in pressure-induced depolarization and myogenic tone in cerebral arteries (40). Interestingly, in the present study, we found that exposure to UTP significantly depolarized the SMCs, but there was no difference between the TRPC6 sense- and antisense-treated groups (Fig. 4A). Furthermore, TRPC6 antisense treatment did not affect the magnitude of constriction after exposure to increasing concentrations of UTP (Fig. 4B), indicating that TRPC6 is not involved in UTP-evoked depolarization or constriction of these arteries.

**Fig. 3.** Suppression of TRPC3 expression with antisense ODNs attenuates UTP-induced membrane depolarization and vasoconstriction. A: summary data showing greater membrane depolarization in sense-treated (n = 6) than in antisense-treated (n = 5) cerebral arteries exposed to increasing concentrations of UTP. Resting membrane potentials (Vm) were −50 ± 1 and −49 ± 2 mV in sense- and antisense-treated arteries, respectively. B: summary data of the decrease in vessel diameter (percent constriction) of TRPC3 sense-treated (n = 4) and antisense-treated (n = 4) arteries exposed to increasing concentrations of UTP. Initial internal diameters were 173 ± 16 and 187 ± 15 μm in sense- and antisense-treated arteries, respectively (no significant difference). Values are means ± SE. *Significant difference (P ≤ 0.05) between sense- and antisense-treated vessels.

**Fig. 4.** Suppression of TRPC6 expression with antisense ODNs does not affect UTP-induced membrane depolarization or vasoconstriction in rat cerebral resistance arteries. A: UTP-induced depolarization in arteries treated with TRPC6 sense (n = 5) or antisense (n = 5) ODNs. B: summary data showing the decrease in vessel diameter (percent constriction) of sense-treated (n = 5) and antisense-treated (n = 5) arteries exposed to increasing concentrations of UTP. Initial diameters were 162 ± 4 and 170 ± 7 μm in sense- and antisense-treated arteries, respectively. Values are means ± SE.
involved in receptor-mediated vasoconstriction. Receptor coupling and activation of TRPC3 have been clearly demonstrated using overexpression approaches in cultured cells (1, 10, 18, 35, 37, 41). Evidence for such coupling has also been obtained for native cell systems. For instance, a TRPC3 channel-dependent current has been observed in rat neonatal pontine neurons after activation of TrkB by brain-derived nerve growth factor (11). TRPC3 channels also contribute to a cation conductance-associated regulation of nuclear factor of activated T cells of skeletal muscle gene expression (29) and the contraction of myometrial smooth muscle during parturition (4, 36). These observations raise the interesting possibility that activation of TRPC3 channels after receptor stimulation may be a rather common occurrence across the diversity of cell types known to express TRPC3 channels.

In the present study, antisense suppression of TRPC3 decreased UTP-induced depolarization of cerebral artery SMCs (Figs. 3 and 4), whereas antisense suppression of TRPC6 was without effect. This suggests that TRPC6 channels are not involved in the pyrimidine receptor-mediated response in these cells. Also, suppression of TRPC3 had no effect on pressure-induced depolarization or the development of myogenic tone by cerebral artery SMCs. Suppression of TRPC6, however, decreases pressure-induced depolarization of cerebral artery SMCs.

DISCUSSION

The major new finding of the present study is that TRPC3 channels contribute to UTP-induced depolarization and constriction of cerebral arterial SMCs. Several observations support this conclusion: 1) TRPC3 mRNA and protein were expressed in SMCs and cerebral arteries of adult rats (Fig. 1). 2) Suppression of TRPC3 channel expression significantly attenuated UTP-induced depolarization and constriction (Fig. 3). 3) Suppression of TRPC3 channel expression nearly eliminated UTP-induced whole cell currents in isolated SMCs (Fig. 5). 4) Inhibition of voltage-dependent L-type Ca\(^{2+}\) channels partially reversed UTP-induced vasoconstriction when TRPC3 channels were present, but not when their expression was suppressed.

Expression of TRPC3 in vascular SMCs. We detected TRPC3 mRNA and protein expression in rat cerebral artery SMCs (Fig. 1, A and B). TRPC3 mRNA and/or protein expression has been detected in other vascular SMCs, including those of the rat aorta (5), rat pulmonary artery (17, 26), mouse portal vein (12), canine renal artery (38), rat preglomerular resistance vessels (5), and rat caudal artery (2). The presence of TRPC3 mRNA and/or protein in a number of vascular SMC types suggests that the contribution of TRPC3 to vascular function may be widespread.

Receptor activation of TRPC3 channels. Our present findings demonstrate for the first time that TRPC3 channels are involved in receptor-mediated vasoconstriction.

Intracellular Ca\(^{2+}\) entry through voltage-dependent Ca\(^{2+}\) channels contributes to UTP-induced vasoconstriction in pressurized rat cerebral arteries. A: original diameter recordings show that 1 μM nisoldipine (NIS), an L-type Ca\(^{2+}\) antagonist, partially reverses constriction induced by UTP in TRPC3 sense-treated, but not antisense-treated, arteries. Paired TRPC3 sense- and antisense-treated arteries were exposed to 1 μM UTP, resulting in a 38% constriction in the sense-treated artery and a 29% constriction in the antisense-treated artery. B: summary of effects of nisoldipine on UTP-induced tone in arteries exposed to TRPC3 sense (n = 10) or antisense (n = 8) ODNs. Initial diameter was 189 ± 15 and 170 ± 15 μm for sense- and antisense-treated arteries, respectively. Values (means ± SE) are expressed as percent dilation, which was calculated as follows: %dilation = ([φ(initial) − φ(UTP)]/[φ(initial)]) × 100. *Significant difference (P ≤ 0.05) between TRPC3 sense- and antisense-treated arteries.
UTP-induced vasoconstriction (Fig. 6). Micromolar concentrations of UTP depolarize arterial SMCs by vasoconstriction (24). Micromolar concentrations of UTP de-

Depolarization and activation of L-type Ca$$^{2+}$$ channels in vascular SMCs is a well-established mechanism of vasooncstruction (24). Micromolar concentrations of UTP depolarize arterial SMCs by $$\sim$$20 mV (Figs. 3 and 4) (19, 39), which is sufficient to open L-type Ca$$^{2+}$$ channels to permit extracellular Ca$$^{2+}$$ influx and constriction (Fig. 6) (15). UTP also activates a TRPC3-mediated inwardly rectifying current in isolated SMCs (Fig. 5). Thus a major conclusion of the present study is that TRPC3 channels are primary mediators of UTP-induced depolarization of cerebral artery smooth muscle. Arterial SMC depolarization is also observed in response to other receptor agonists, such as norepinephrine (8, 23, 25), histamine (3, 7), and 5-hydroxytryptamine (23), indicating that SMC depolarization is an important component of receptor-mediated vasooncstruction. It will be interesting to determine whether TRPC3 mediates depolarization induced by these and other vasooncstructor agonists.

Although antisense suppression of TRPC3 channels significantly attenuated an inward current and SMC depolarization in response to UTP, some depolarization was still detectable in the TRPC3 antisense-treated arteries. This was likely due to incomplete TRPC3 channel suppression (Fig. 2) and/or UTP-mediated activation of other depolarizing mechanisms (9, 19, 28, 32, 39). Furthermore, inhibiting L-type Ca$$^{2+}$$ channels only partially attenuated UTP-induced vasoconstriction (Fig. 6). This illustrates that alternative constrictor mechanisms, such as release of Ca$$^{2+}$$ from the sarcoplasmic reticulum (13), altered myofilament Ca$$^{2+}$$ sensitivity (31), or inhibition of myosin light chain phosphatase (6, 30), also regulate vasoconstriction induced by UTP. The relative contributions of these signaling modalities to the overall contractile response of vascular smooth muscle remain to be determined.

Summary. TRPC3 channels can be added to the list of membrane channels that contribute to the ion fluxes controlling arterial diameter. TRPC3 channels are distinctly involved in UTP-induced depolarization and constriction of arterial smooth muscle, whereas TRPC6 channels contribute to the arterial myogenic response. The unique and differential activation of these ion channels by various excitatory stimuli could have important implications concerning the development of new therapeutic strategies targeted to specific vascular SMC constrictor mechanisms in vascular disease states.

ACKNOWLEDGMENTS

We thank Katherine Lutz for technical assistance.

REFERENCES

1. Basora N, Boulay G, Bilodeau L, Rousseau E, and Payet MD. 20-

References to the authors' work have been updated to reflect the latest and most relevant research. This text is a detailed explanation of the mechanisms underlying the role of TRPC3 channels in cerebral artery smooth muscle, highlighting the importance of these channels in regulating arterial diameter and vasoconstriction, and the implications for potential therapeutic strategies.


