20-HETE inotropic effects involve the activation of a nonselective cationic current in airway smooth muscle

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Cloutier, Martin, Shirley Campbell, Nuria Basora, Sonia Proteau, Marcel D. Payet, and Eric Rousseau. 20-HETE inotropic effects involve the activation of a nonselective cationic current in airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 285: L560–L568, 2003. First published May 16, 2003; 10.1152/ajplung.00381.2002.—20-Hydroxyeicosatetraenoic acid (20-HETE) controls several mechanisms such as vasoactivity, mitogenicity, and ion transport in various tissues. Our goal was to quantify the effects of 20-HETE on the electrophysiological properties of airway smooth muscle (ASM). Isometric tension measurements, performed on guinea pig ASM, showed that 20-HETE induced a dose-dependent inotropic effect with an EC50 value of 1.5 μM. This inotropic response was insensitive to GF-109203X, a PKC inhibitor. The sustained contraction, requiring Ca2+ entry, was partially blocked by either 100 μM Gd3+ or 1 μM nifedipine, revealing the involvement of noncapacitative Ca2+ entry and L-type Ca2+ channels, respectively. Microelectrode measurements showed that 3 μM 20-HETE depolarized the membrane potential in guinea pig ASM by 13 ± 2 mV (n = 7), as did 30 μM 1-oleoyl-2-acetyl-sn-glycerol. Depolarizing effects were also observed in the absence of epithelium. Patch-clamp recordings demonstrated that 1 μM 20-HETE activated a nonselective cationic inward current that may be supported by the activation of transient receptor potential potential channels. The presence of canonical transient receptor potential receptor potential mRNA was confirmed by RT-PCR in guinea pig ASM cells.

20-hydroxyeicosatetraenoic acid; calcium; isometric tension; membrane potential; transient receptor potential; nonselective cationic current

THE AIRWAY SMOOTH MUSCLES (ASM) contract in response to various agonist stimuli, involving the activation of G protein-coupled receptors (16). These inotropic responses are modulated by membrane depolarization, which relies on ion channel activation and conductance changes (19). These are mediated by various means, including lipidic activation by arachidonic acid (AA) metabolites. 20-Hydroxyeicosatetraenoic acid (20-HETE) is an AA metabolite produced by cytochrome P-450 (CYP-450) ω-hydroxylases. The CYP-450 enzymes are predominantly detected in liver (26), heart (38), vasculature, gastrointestinal tract, kidney (32), and lung (40). In guinea pig, 20-HETE induces an increase of ASM basal tone (39) and may have additive effects on sustained contraction of ASM preconstricted using carbachol (CCh). In contrast, 20-HETE relaxes rabbit (18) and human (41) bronchi preconstricted with histamine or KCl. It has also been reported that 20-HETE causes contraction of vascular smooth muscle (VSM) in various species, including cat, dog, and rat, although it causes relaxation of human and rabbit VSM in pulmonary circulation (28). These relaxant effects are inhibited by indomethacin, a cyclooxygenase (Cox) inhibitor, or by removal of the endothelium lining (18). However, little is known about the mechanisms that lead to contraction in guinea pig ASM. Experiments involving exogenous addition of 20-HETE might help to discriminate its mode of action on electrophysiological parameters. In the sustained portion of a contraction of bovine tracheal smooth muscle strips induced by muscarinic stimulation, the majority of Ca2+ entry from the extracellular medium is not mediated via voltage-operated Ca2+ channels (34) but possibly via a nonspecific cation channel, such as the transient receptor potential (TRP) channels (4, 8). In VSM, it has been shown that the mechanism that leads to activation of canonical transient receptor potential potential (TRPC) 6 is associated with Gq-coupled receptors, such as the α1-adrenoceptor, which activate phospholipase C (PLC; see Ref. 6). The PLC produces diacylglycerol (DAG) and inositol trisphosphate from phosphatidylinositol 4,5-bisphosphate. DAG, via a protein kinase C (PKC)-independent mechanism, plays a central role in the activation of TRPC6 (17, 21, 22). It has been shown that AA, produced by DAG lipase from DAG, activates a noncapacitative Ca2+ entry in A7r5 smooth muscle cells stimulated with low concentrations of vasopressin (6). Moreover, Welsh et al. (37) show that suppressing the protein expression of TRPC6 using specific antisense oligonucleotides reduced the current density of a major cation current in resistance artery smooth muscle, which suggests that a nonselective cationic current might be important in smooth muscle cell electrophysiology (30).
The aim of the present study was to test whether or not 20-HETE, an AA metabolite, can induce ASM contraction and modulate membrane potential in relation to ionic conductance activation. We assessed the mechanical and electrophysiological effects of 20-HETE on ASM at the tissue and cellular levels, respectively. We used the following three complementary experimental approaches: 1) isometric tension measurements on guinea pig ASM induced by 20-HETE, 2) membrane potential measurements using the classical microelectrode technique and quantification of the pharmacological effects of 20-HETE and 1-octeyl-2-acetyl-sn-glycerol (OAG), and 3) patch clamp to assess the effects of 20-HETE on macroscopic currents. Our results show that 20-HETE induced concentration-dependent contractions of ASM, depolarized membrane potential, and activated a nonselective cationic current across the surface membrane of ASM cells. Part of this work has been communicated elsewhere in abstract form (9).

MATERIALS AND METHODS

Isometric tension measurements. The mechanical effects of 20-HETE were measured on helically cut trachea and main bronchi taken from albino guinea pigs (weighing 250–300 g; Hartley), as previously reported (5, 7, 10). A Krebs solution, containing (in mM) 118.1 NaCl, 4.7 KCl, 1.2 MgSO4·7H2O, 1.2 KH2PO4, 25 NaHCO3, 2.5 CaCl2, and 11.1 glucose, pH 7.4, was used as physiological medium. The effects of 20-HETE on basal tone were measured using a Grass polygraph. The inotropic effects of 20-HETE were quantified and normalized to those induced by 0.1 mM 7,8-dihydroxy-8-oxo-2′-deoxyguanosine (OAG), and 3 μM theophylline. We also measured OAG-induced contractions at 1 μM 20-HETE to assess the relative contributions of OAG to the development of 20-HETE responses. The tissues were superfused at a constant flow rate of 2 ml/min with standard Krebs solution and allowed to equilibrate for 20 min followed by another 20 min with 5 μM theophylline to prevent spontaneous smooth muscle contractions at the time of impalements. Unused tissues were kept at 4°C in oxygenated Krebs solution for several hours. Membrane potential was measured using conventional intracellular borosilicate microelectrodes filled with 3 M KCl and resistance ranging from 30 to 50 MΩ. The microelectrodes were connected via an Ag/AgCl2 pellet to a headstage of an amplifier mounted on a N13004 micro-manipulator from Narishige (Tokyo, Japan). Measurements were performed with a KS-700 amplifier from World Precision Instruments (Sarasota, FL). Electrical signals were monitored continuously on a TDS 310 oscilloscope (Tektroniks, Beaverton, OR). The membrane potential was digitized and recorded using a Digidata 1200B interface and Axoscope 7.0 software from Axon Instruments (Union City, CA). Data were stored on disk for further analysis. Electrophysiological measurements were also performed on albino rabbits (1.5–2.5 kg) and mongrel dog tracheas. In these experiments, a Tyrode solution containing (in mM) 136 NaCl, 4 KCl, 1.8 CaCl2, 0.5 MgCl2, 0.35 Na2HPO4, 12.5 NaHCO3, and 11 dextrose, pH 7.4, was used.

Patch-clamp recording. Whole cell currents were measured at room temperature from guinea pig ASM cells (15) using fire-polished patch pipettes (3–6 MΩ) with uncompensated series resistance. Currents were recorded with an Axopatch amplifier (Axon Instruments), controlled by homemade software. The standard holding potential was −40 mV, and membrane currents were filtered at 500 Hz and acquired at 1,000 Hz. The standard intracellular solution contained (in mM) 140 NaCl, 1.8 CaCl2, 12.5 MgCl2, 15 HEPES, 0.3 ATP, and 0.03 GTP, pH 7.2 (calculated free internal Ca2+: 100 nM). The standard bath solution contained (in mM) 140 NaCl, 1.8 CaCl2, 12.5 MgCl2, 15 HEPES, and 10 glucose, pH 7.4. The final concentration of 1 μM 20-HETE was added to the perfusion solution containing (in mM) 145 NaCl, 2.5 EGTA, and 5 HEPES, pH 7.4, by a local perfusion system (Perfusion fast-step; Harvard Apparatus, Holliston, MA). Depolarizing voltage ramps were applied at a rate of 100 mV/s, from −100 to +60 mV.

Cell culture. Male or female albino guinea pigs (weighing 350–450 g; Hartley) were anesthetized by a lethal dose of pentobarbital sodium (50 mg/kg ip) and killed by abdominal exsanguinations. The trachea was excised aseptically and placed immediately on ice in sterile Krebs solution (see composition above). Under sterile conditions, and on ice, the trachea was cut free of excess tissue and cut longitudinally on the opposite side of the smooth muscle. The epithelial cells were removed mechanically with a sterile cotton swab. The smooth muscle tissue was minced, washed in MEM containing 200 μM free Ca2+, and centrifuged at 80 g for 1 min. The pellet was resuspended and dissociated in 200 μM Ca2+ MEM with 640 U/ml collagenase (type IV), 10 U/ml elastase (type IV), and 20 μg/ml DNase (type I), all from Sigma-Aldrich (Oakville, ON, Canada). The tissue was digested in a cell incubator at 37°C for 3 × 20 min with agitation at each step. The cell suspension was then filtered through a 100-μm Nylon Cell Strainer, and the filtrate was washed with 900 μM Ca2+ MEM. The cells were centrifuged at 80 g for 10 min, and the pellet was resuspended in 1 ml Opti-MEM supplemented with 2% FBS and 1% penicillin-streptomycin. The cells were plated in 35 mm-dishes with ~104 cells for each dish, and, after 30 min incubation at 37°C, the dishes were completed with 2 ml Opti-MEM.

Molecular biology. Guinea pig ASM cells were isolated and cultured on plastic dishes as described above. Total RNA was extracted using the RNaqueous method according to the manufacturer (Ambion, Austin, TX). RNA (5 μg) was used for each preparation and was reverse transcribed into first-strand cDNA, oligo(dT) (5 units), dNTP (10 mM) from Amer sham-Pharmacia Biotech (Piscataway, NJ), DTT (0.1 M), Moloney murine leukemia virus, and RNasin (all from Promega, Madison, WI). cDNA was amplified for each PCR reaction by using specific primers based on published sequences for TRPC1, -3, -4, and -5 (13) or primers designed based on the GenBank sequence for TRPC6. The sequences of the primer were as follows: 1) mouse (m) TRPC1: sense 5′-CAAGATTTTGAGGAATATTCTCG-3′ and antisense 5′-TTTCTATTGATTTGCTAT-3′; 2) human (h) TRPC3: sense 5′-TACTCTTCGTTGTGCTCCAATATG-3′ and antisense 5′-CTCTCAGAGGCTCTCCCTCTCGTGC-3′; 3) mTRPC4: sense 5′-GCTGCATATACCTCTGGGAGGATGC-3′ and antisense 5′-AAGCT-TTGTGCAGCAAATTTCCATC-3′; 4) mTRPC5: sense 5′-ATCTACTGCG-CTGACTCTAGGGC-3′ and anti-
sense 5’-CAGCATGATCGGCAATGAGCTG-3’; and 5) rat TRPC6: sense 5’-AACAAAAGCATGACTCTCCAG-3’ and antisense 5’-AAGGAGCA-CACCATATATGAGA-3’. GAPDH was used as a control of RNA integrity. Amplification was performed using Taq polymerase on a Perkin-Elmer amplification system for 34 cycles consisting of 30 s at 94°C, 60 s at 55°C, and 2 min at 72°C for extension for all samples. Products were loaded on a 2% agarose gel in Tris-acetate-EDTA buffer with 0.1 μg/ml ethidium bromide. After electrophoresis, the gel was scanned by a Fluorimag (Alpha Innotech, San Leandro, CA).

Drugs and chemical reagents. 20-HETE from Cayman Chemical (Ann Arbor, MI) was dissolved in 100% ethanol and stored as 1 mM stock solutions. The vehicle was tested separately at the maximal concentration used in the presence of active compound. CCh, nifedipine, and iberiotoxin were purchased from Sigma (St. Louis, MO). Gadolinium chloride was purchased from ICN Biomedicals (Cleveland, OH), and OAG was from Calbiochem (San Diego, CA). FBS, penicillin-streptomycin, and all cell media were purchased from GIBCO Invitrogen (Burlington, ON, Canada).

Data analysis and statistics. Results were expressed as means ± SE; n indicates the number of experiments. Statistical analyses were performed using either paired or unpaired Student’s t-tests, as well as ANOVA. Values of P < 0.05 were considered significant. Data curve fittings were performed using SigmaPlot 8.0 (SPSS-Science, Chicago, IL). The concentration-response curve was fitted to the equation

\[ C = C_{max}(X^{H_1}) / (EC_{50}^{H_1} + X^{H_1}) \]  

where C and C_{max} are the amplitude of contraction, X is the concentration of 20-HETE, EC_{50} is the concentration of 20-HETE that produces half-maximal amplitude of contraction, and H_1 is the Hill coefficient. Patch-clamp analysis was performed with homemade software.

**RESULTS**

Tension measurements. The addition of cumulative concentrations of 20-HETE produced sustained contractions that were reversed by washing with fresh, oxygenated (95% O_2-5% CO_2) Krebs solution (Fig. 1A). In guinea pig preparations, 20-HETE induced reversible, concentration-dependent positive inotropic responses. Vehicle alone, ethanol, was tested, and the various quantities used were shown to have no effects on the resting tone of guinea pig ASM (Fig. 1B). To account for biological variations, the data were normalized using the amplitude of contraction induced by 0.1 μM CCh obtained for each preparation at the beginning of all experiments. 20-HETE inotropic effects were expressed as a percentage (%) of the contraction induced by 0.1 μM CCh, a concentration that was previously determined as the EC_{50} on guinea pig ASM (3). Figure 1C shows the dose-response curve for 20-HETE. This eicosanoid-induced tonic concentration-dependent response was undetected below 0.03 μM and saturating above 10 μM. An EC_{50} value of 1.5 μM and a Hill coefficient of 0.77 were obtained by fitting Eq. 1 to the experimental data. These results suggest the presence of a receptor for 20-HETE that remains to be characterized (11).

Effects of Ca^{2+} release and Ca^{2+} entry on the inotropic effect of 20-HETE. Figure 2A shows the sustained contractions induced by 20-HETE in the presence of Krebs solution containing 2.5 mM Ca^{2+}. In contrast, 20-HETE induced only a transient contraction in Ca^{2+}-free Krebs solution (Fig. 2B). When tension returned to basal levels, addition of 2.5 mM CaCl_2 induced a large tension increase. This effect was fully reversible upon washout of 20-HETE (Fig. 2B). To test the relative contribution of nonselective cation channels and voltage-dependent L-type Ca^{2+} channels, Gd^{3+}, which had no effect on the resting tone (data not shown), and nifedipine were used sequentially. Gd^{3+} (100 μM), a nonspecific cation channel blocker, has been reported to block activities of the TRPC6 implicated in the control of myogenic tone in VSM (21, 37). Gd^{3+} (100 μM) induced relaxation of guinea pig ASM precon-
tracted with 1 μM 20-HETE (Fig. 2, C and D), whereas 1 μM nifedipine, used to block voltage-dependent L-type Ca²⁺ channels, relaxed the remaining tension. Table 1 summarizes the results of two series of complementary and comparative experiments where the relaxing effects of 1 μM nifedipine were measured on 20-HETE (n = 12)- and KCl (n = 4)-induced tension in guinea pig ASM. Nifedipine was much more effective in inhibiting KCl-induced responses than those induced by 20-HETE. However, 100 μM Gd³⁺ had no effect on high KCl-induced tension (data not shown). In contrast, the relaxing effect of 1 μM 20-HETE (Fig. 2B) was abolished by the addition of 100 μM Gd³⁺ (n = 12). Together, these results suggest that both L-type Ca²⁺ channels and nonselective cation channels are involved in the pharmacomechanical coupling induced by 20-HETE.

**Table 1. Average relaxing effects of nifedipine on 20-HETE and KCl-induced tensions in guinea pig airway smooth muscle**

<table>
<thead>
<tr>
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<th>Tension Developed, mg</th>
<th>Relaxing Effect of 1 μM Nifedipine, %</th>
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<tbody>
<tr>
<td>20-HETE (1 μM)</td>
<td>12</td>
<td>155.8 ± 26.9</td>
</tr>
<tr>
<td>KCl (80 mM)</td>
<td>4</td>
<td>734.4 ± 35.9</td>
</tr>
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Values are means ± SE; n, no. of guinea pigs. 20-HETE, 20-hydroxyeicosatetraenoic acid. Data were obtained from experiments performed on guinea pig trachea and external main bronchi.

Fig. 2. Roles of intracellular Ca²⁺ release and Ca²⁺ entry in guinea pig ASM upon 20-HETE stimulation on intact epithelium. A: sustained tension induced by 20-HETE in normal Krebs solution. B: transient effect of 20-HETE in Ca²⁺-free Krebs solution, followed by tonic tension increase upon addition of 2.5 mM CaCl₂ in the experimental chamber. C: relaxant effect of 100 μM Gd³⁺ after precontraction with 1 μM 20-HETE. D: relaxing effects of 100 μM Gd³⁺ and 1 μM nifedipine after precontraction with 1 μM 20-HETE. All of these effects were reversible after washout.

**Effect of 20-HETE on ASM membrane potential.** The effects of 20-HETE on the membrane potential of...
guinea pig ASM cells were assessed after microelectrode impalement and continuous recordings. The muscle strip was superfused with a physiological solution for several minutes, and then micromolar concentrations of 20-HETE were applied. After a stable membrane potential of \( -60 \) mV was obtained in this sample where the epithelium had been kept intact, \( 3 \) \( \mu \)M 20-HETE was superfused, and depolarization was recorded after a short delay, as shown in Fig. 4A. 20-HETE (3 \( \mu \)M) depolarized the membrane potential of ASM by \( 13 \) \( mV \) (n = 7), an effect that was fully reversible within a few minutes. The mean electrophysiological effect of 20-HETE on guinea pig and on tissue recovery are shown in Fig. 4B.

Since it was reported that the effects of 20-HETE could be different from one species to the next, complementary experiments were performed on rabbit and canine tissues. Because it is quite difficult to maintain microelectrode impalements for long periods, we used a multi-impalement method during sequential changes in experimental conditions. For instance, in rabbit ASM, 1 \( \mu \)M 20-HETE induced an average depolarization of \( 15 \pm 2 \) mV (n = 7), an effect that was fully reversible within a few minutes. The mean electrophysiological effect of 20-HETE on guinea pig ASM and on tissue recovery are shown in Fig. 4B.

Fig. 3. Protein kinase C (PKC) is not involved in the 20-HETE inotropic response. A: paired recordings of 1 \( \mu \)M 20-HETE challenges in the absence (control) and after GF-103209X preincubation on guinea pig ASM. B: quantitative analysis of the average responses for paired challenges. Reported values are means \( \pm \) SE (n = 12). Paired t-test shows that the difference was not statistically significant.
Effect of 20-HETE on macroscopic nonselective currents in guinea pig ASM cells. Patch-clamp experiments were performed on primary cultured ASM cells from guinea pig, as described in MATERIALS AND METHODS. 20-HETE (1 \mu M) activates a nonselective cationic current under experimental conditions used to measure the current supported by TRPC channel proteins (Fig. 6A). The current (I) activated by 20-HETE during a voltage ramp (-100 to +60 mV) was visualized by data subtraction [Fig. 6B; I_{b-a} (20-HETE) = I_{b} (total) - I_{a} (control)], as reported in Fig. 6A. Hence, the increase in current density generated by 20-HETE was calculated and reported in Fig. 6C. This inward current activated by 20-HETE was likely generated by TRPC channel openings. Furthermore, it has been reported recently by our group that the current supported by the TRPC6 channel proteins overexpressed in HEK293 cells was increased by 20-HETE and OAG and inhibited by either Gd^{3+} or N-methyl-d-glucamine (1).

Expression of TRPCs in guinea pig ASM. RT-PCR was used to identify the types of TRPC channels present in guinea pig ASM cells. TRPC1, -3, -4, -5, and -6 were amplified using specific primers (see Molecular biology) to visualize which forms are present in this tissue. Our results showed that TRPC3, -4, -5, and -6 were expressed in guinea pig ASM cells, but not TRPC1, although the signal was present in brain tissues used as a control (Fig. 6D).

DISCUSSION

This work provides the first direct evidence that the mode of action of 20-HETE on ASM involves the activation of a nonselective cationic conductance, in addition to L-type Ca^{2+} channels. Our results also show that 20-HETE induces concentration-dependent tension increases in guinea pig ASM. Regardless of the Ca^{2+} released from intracellular stores during the contraction, 20-HETE also induced Ca^{2+} entry during the development of the tonic response. Moreover, 20-HETE depolarized the membrane of guinea pig ASM cells in a similar manner as OAG in canine ASM cells. Hence, OAG has been shown to activate nonselective currents in other tissues (35), and, based on the similar effects of OAG and 20-HETE on membrane potential, it was suggested that the mechanism of action of 20-HETE could also involve nonselective cationic currents generated by TRPC channels. This working hypothesis has now been tested in native ASM cells. Our results correlate well with other data recently reported in the literature on VSM (6, 30, 37) and further support the physiological role of nonselective cationic currents in the tonic responses triggered by eicosanoids.  

Pharmacological responses to 20-HETE. Because of the biological variability of the tonic responses after eicosanoid challenge on airway tissues, we measured the positive inotropic effects of 20-HETE on guinea pig ASM and normalized the mechanical responses as a percentage of the response induced by 0.1 \mu M CCh on the same tissue. Concentrations of 20-HETE >0.03 \mu M induced tonic concentration-dependent responses, saturating above 10 \mu M. In contrast, 20-HETE has been reported to relax rabbit bronchi preconstricted with histamine or KCl (18) and human bronchi preconstricted with histamine (41). The relaxant effect of 20-HETE on these ASM tissues was blocked by indomethacin or after epithelium removal. These results indicate that the effects of 20-HETE are species dependent and could be related to differential expression profiles of Cox isozymes and metabolite production. The concentration-response curve performed on guinea pig ASM revealed an EC_{50} value of 1.5 \mu M for 20-HETE. In contrast, other related eicosanoids, such as the epoxyeicosatrienoic acid regioisomers, which are produced by the CYP-450 epoxygenase, were shown to trigger relaxing and hyperpolarizing responses in guinea pig ASM preparations (5, 10).

The positive inotropic effects of 20-HETE mobilize Ca^{2+} from intracellular stores, as attested by the transient responses observed in the absence of extracellular Ca^{2+}. However, Ca^{2+} entry was necessary to trigger and maintain the sustained inotropic responses that occurred upon addition of 2.5 mM extracellular Ca^{2+} concentration (Fig. 2B). It was already reported...
that, in bronchia, Ca^{2+} entry could be the result of activation of the capacitative Ca^{2+} entry after depletion of intracellular Ca^{2+} stores upon ACh stimulation (33). The tonic response induced by 20-HETE was partially relaxed by 100 \mu M d3, a concentration known to block noncapacitative Ca^{2+} entry (25), suggesting a putative role for this molecular process. Taking into account that nifedipine alone partially relaxes the tonic responses induced by 20-HETE (Table 1), it was suggested that L-type Ca^{2+} channels play a complementary role in ASM contraction (20). Indeed, pharmacological maneuvers and electrophysiological experiments in animal and human ASM have demonstrated the existence of dihydropyridine-sensitive Ca^{2+} currents in these samples (24). On the other hand, it has been reported that 20-HETE induced an increase in intracellular free Ca^{2+} concentration with a concomitant activation of L-type Ca^{2+} channels in VSM (14). Thus 20-HETE challenges could be involved in the activation of Ca^{2+} selective channels and a nonselective cationic pathway. In ASM cells, this process would be independent of PKC activation, since the inotropic effect of 20-HETE was not modified in the presence of PKC inhibitor according to the results reported in Fig. 3, A and B.

**Electrophysiological effects of 20-HETE.** Our results show that 20-HETE depolarized the membrane of guinea pig ASM cells and that this effect was fully
reversible. This behavior was also observed in rabbit ASM in the presence and in the absence of the epithelial layer, suggesting that 20-HETE interacted directly with membrane components of ASM cells. Similar results were observed in canine renal arteries, where 20-HETE is associated with a 10-mV depolarization of the membrane potential (23). OAG is a stable and membrane-permeable analog of DAG that has been reported to activate the TRPC3/6/7 channels in Jurkat cells and human peripheral blood T lymphocytes (17, 27). Our results indicate as well that 30 μM OAG depolarizes ASM cells and that this effect was reversible (Fig. 5B). It was previously shown that OAG also depolarizes the membrane of T lymphocytes in Ca2+-free media (12).

The depolarizing effects of 20-HETE and OAG on ASM membrane potential could be explained by the activation of an inward cationic current. Hence, the electrophysiological effects of 20-HETE were sensitive to Gd3+, a blocker of nonselective conductances. Thus we tested the effects of 20-HETE on the macroscopic inward currents, which under our experimental conditions may be generated by activation of TRPC channels in smooth muscle, as previously reported by other laboratories working on various biological structures (6, 21, 30). Addition of exogenous 20-HETE consistently activated nonselective cationic currents. Such currents had already been reported to be activated by DAG (2, 36) and OAG (17, 27). The activation of an inward cationic current might partially explain the depolarization induced by 20-HETE and OAG on ASM. These observations do not rule out the putative contribution of other pharmacological (via eicosanoid receptors or lipid-gated channels) and biochemical (via the activation of intracellular cascades) pathways. However, they provide evidence that this hydrophobic eicosanoid, generated in vivo by CYP-450/ω-hydroxylases upon AA release after phospholipase A2 activation, might regulate surface membrane conductances, which are likely involved in the control of the basal ASM tone (30). A role of TRPC channels in controlling the VSM myogenic tone has already been demonstrated by two independent groups (6, 37). However, to date, the role of TRP channels and more specifically the TRPC6 isoform has not been precisely forecast in ASM, except by Snetkov et al. (31), who had envisioned their implication on bronchoactive leukotriene D4 stimulation. Recently, in a set of key experiments, our group has demonstrated that exogenous 20-HETE and OAG, in the micromolar concentration range, activate nonselective cationic currents in HEK293 cells stably overexpressing TRPC6 (1). The direct implication of TRPC6 in the mode of action of 20-HETE in ASM cells is plausible since the addition of 100 μM Gd3+, which has been reported to block the noncapacitative entry of Ca2+ (25) and more directly the TRPC6 channel isoform (21), partially relaxes its positive inotropic effect as shown and discussed above. Although we have not tested the effects of 20-HETE on TRPC activity in an overexpression system, our present results reveal the presence of the TRPC3, -4, -5, and -6 mRNA in guinea pig ASM cells (9).

In summary, 20-HETE induces concentration-dependent positive tonic responses in guinea pig ASM. This tonic response is triggered by intracellular Ca2+ release and is maintained by Ca2+ entry, the latter being related to a depolarization of the membrane potential as shown on the same preparation. The following two currents are likely to be involved in this process: voltage-dependent L-type Ca2+ currents and nonselective cationic currents. Although this hypothesis remains to be assessed, our results suggest that TRPC channels are likely to support some of the latter currents, even if lipid-gated channels related to the vanilloid receptor family cannot be disregarded (4).

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DISCLOSURES

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