Lysophosphatidyl choline modulates mechanosensitive L-type Ca\textsuperscript{2+} current in circular smooth muscle cells from human jejunum

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lysophosphatidyl choline modulates mechanosensitive L-type Ca\textsuperscript{2+} current in circular smooth muscle cells from human jejunum. Am J Physiol Gastrointest Liver Physiol 296: G833–G839, 2009. First published January 29, 2009; doi:10.1152/ajpgi.90610.2008.—The L-type Ca\textsuperscript{2+} channel expressed in gastrointestinal smooth muscle is mechanosensitive. Direct membrane stretch and shear stress result in increased Ca\textsuperscript{2+} entry into the cell. The mechanism for mechanosensitivity is not known, and mechanosensitivity is not dependent on an intact cytoskeleton. The aim of this study was to determine whether L-type Ca\textsuperscript{2+} channel mechanosensitivity is dependent on tension in the lipid bilayer in human jejunal circular layer myocytes. Whole cell currents were recorded in the amphotericin-perforated-patch configuration, and lysophosphatidyl choline (LPC), lysophosphatidic acid (LPA), and choline were used to alter differentially the tension in the lipid bilayer. shear stress (perfusion at 10 ml/min) was used to mechanostimulate L-type Ca\textsuperscript{2+} channels. The increase in L-type Ca\textsuperscript{2+} current induced by shear stress was greater in the presence of LPC (large head-to-tail proportions), but not LPA or choline, than in the control perfusion. The increased peak Ca\textsuperscript{2+} current also did not return to baseline levels as in control conditions. Furthermore, steady-state inactivation kinetics were altered in the presence of LPC, leading to a change in window current. These findings suggest that changes in tension in the plasmalemmal membrane can be transmitted to the mechanosensitive L-type Ca\textsuperscript{2+} channel, leading to altered activity and Ca\textsuperscript{2+} entry in the human jejunal circular layer myocyte.

calcium channel; gastrointestinal; lipid bilayer tension; shear stress; lysophosphatidic acid

THE CONCEPT OF SMOOTH MUSCLE mechanosensitivity of the gut was first introduced in the 1950s with the discovery that smooth muscle can respond to stretch in the absence of extrinsic neural input (2). Later, the development of patch-clamp techniques led to the discovery of mechanosensitivity as an inherent property of some ion channels (15, 26), suggesting that these ion channels transduce mechanosensitivity in a variety of cells. Indeed, cells containing mechanosensitive ion channels are able to directly sense and then respond to mechanical forces (26, 40) by altering the flow of ions across the cell membrane.

The smooth muscle of the gut, like other smooth muscle, requires entry of Ca\textsuperscript{2+} for contraction. The increase in intracellular Ca\textsuperscript{2+} that precedes contraction is due, in part, to Ca\textsuperscript{2+} entry from outside the cell and, in part, to release of Ca\textsuperscript{2+} from intracellular stores. These processes are interdependent, because Ca\textsuperscript{2+} release from intracellular stores relies largely on entry of extracellular Ca\textsuperscript{2+}, dubbed Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (20). The major pathway for extracellular Ca\textsuperscript{2+} into the intestinal smooth muscle cell is the L-type Ca\textsuperscript{2+} channel (11), as supported by evidence that L-type Ca\textsuperscript{2+} channel blockers abolish effectively inward Ca\textsuperscript{2+} current.

Intestinal smooth muscle L-type Ca\textsuperscript{2+} channels are gated by voltage and a number of chemical mediators (7, 22, 41, 44, 53). The channels are also mechanosensitive, and increased peak current through the channel is observed with mechanical perturbation of the cell membrane (9, 18, 24). This mechanosensitivity is an inherent property of the pore-forming unit of the channel (24). Current models of ion channel mechanosensitivity ascribe mechanosensitivity through two major mechanisms. One mechanism, transfer of force through connections between the cytoskeleton and the ion channel, is operative in human gastrointestinal smooth muscle, where Na\textsubscript{v}1.5 mechanosensitivity is abolished by disruption of the actin cytoskeleton without an effect on L-type Ca\textsuperscript{2+} channel mechanosensitivity (37), suggesting that another mechanism must be operative for the latter. Transfer of force from the lipid bilayer to the ion channel is the second proposed mechanism for ion channel mechanosensitivity (40). This model predicts that the composition, and hence the ability to impart force to the channel, of the lipid bilayer would alter mechanosensitivity. The aim of the present study was to determine whether L-type Ca\textsuperscript{2+} channel mechanosensitivity is dependent on the characteristics of the lipid bilayer by altering the composition of the bilayer. To differentially alter the tension within the lipid bilayer, we used lysophosphatidyl choline (LPC), which has a polar head and single fatty acid chain, lysophosphatidic acid (LPA), which has a fatty acid tail but no polar head, and choline, which has a polar head but no fatty acid tail. We found that LPC, but not LPA or choline, altered the mechanosensitive response and steady-state kinetics of the channel, suggesting that tension in the lipid bilayer can directly affect L-type Ca\textsuperscript{2+} channel activity.

METHODS

Dissociation of human jejunal circular smooth muscle cells. Human jejunal tissue was obtained as surgical waste tissue from bariatric surgery performed for morbid obesity in otherwise healthy subjects. Tissue specimens were harvested directly into chilled buffer solution with warm ischemia times of <30 s. Single, isolated circular smooth muscle cells were obtained from the human jejunal specimens as described previously (38). The freshly isolated cells were used for electrophysiological recordings within 6 h of dissociation.

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Electrophysiology. Whole cell patch-clamp recordings were obtained by using Kimble KG-12 glass pulled on a P-97 puller (Sutter Instruments, Novato, CA). Electrodes were coated with R6101 (Dow Corning, Midland, MI) and fire polished to a final resistance of 3–5 MΩ. Currents were amplified, digitized, and processed using an Axopatch 200A or 200B amplifier, a Digidata 1322A, and pCLAMP 9 software (Molecular Devices, Union City, CA). Data were filtered at 5 kHz with an eight-pole Bessel filter. The junction potential between pipette solution and bath solution was adjusted electronically to zero. Access resistance was recorded, and data were used only if access varied <5 MΩ throughout the experiment. Records were obtained in amphotericin-perforated, whole cell patch-clamp mode to limit rundown of the L-type Ca²⁺ current. Cells were held at −100 mV and pulsed (for 50 ms each) from −80 to +35 mV in 5-mV steps. The interpulse start-to-start time was 1 s to allow complete recovery from inactivation. All electrophysiological experiments were carried out at room temperature (20°C). Human jejunal circular layer myocytes express three dominant inward currents: a current carried by an L-type Ca²⁺ channel (CaV1.2) (24), a current carried by an Na⁺ channel (NaV1.5) (17, 31), and a nonselective current. Under physiological recording conditions, the L-type Ca²⁺ current is discernable in 85% of cells and the Na⁺ current in 89% of cells (38). Both are mechanosensitive but can be teased apart, because >90% of Na⁺ current inactivates <20 ms after initiation of a depolarizing pulse. Data presented in the figures show Na⁺ and Ca²⁺ currents. On average, the peak inward Na⁺ current is 87% greater than Ca²⁺ current at a holding voltage of −100 mV when both currents are present (−127 ± 8 vs. −68 ± 3 pA, n = 242, P < 0.01).

Mechanical activation of L-type Ca²⁺ channels. Perfusion of normal Ringer extracellular fluid at a rate of 10 ml/min for 1 min was used as the mechanical stimulus, as reported previously (24). The experimental protocol used to examine changes in peak inward current for the mechanosensitive L-type Ca²⁺ current was designed with an internal control (Fig. 1). Up to 10 min were allowed for amphotericin B to diffuse to and perforate the cell membrane. An additional 6 min were allowed for run-up until a stable baseline of Ca²⁺ current was reached. After 1 min of control perfusion with Ringer solution at 10 ml/min, Ca²⁺ current was allowed to return to baseline for 8 min. Then 100 µl of Ringer solution (control) or a solution of 100 µl of LPC (10 µM final concentration), LPA (10 µM final concentration), or choline (10 µM final concentration) was pipetted directly into the still bath (to prevent mecanosactivation) and allowed 2 min to equilibrate. Subsequently, a second perfusion was performed at the same rate and duration as the first, and a final 8-min postperfusion follow-up period allowed enough time to check for full reversibility of mecanostimulated Ca²⁺ current. Current was measured at 1- to 2-min intervals during the course of each experiment.

Steady-state kinetics for activation and inactivation were calculated before and after the separate applications of LPC, LPA, and choline into the extracellular solution. The pulse protocol was similar to that described previously (11); however, in the present experiments, cells were held at −100 mV to allow better separation of the Ca²⁺ and Na⁺ currents (17).

Drugs and solutions. The pipette solution contained (in mM) 130 Cs⁺, 125 methanesulfonate, 20 Cl⁻, 5 Na⁺, 5 Mg2⁺, 5 HEPES, and 2 EGTA (with final pH adjusted to 7.35 with CsOH). Amphotericin B (Sigma-Aldrich, St. Louis, MO) was dissolved in DMSO and suspended in the pipette solution. The final concentration of DMSO did not exceed 0.1%. The bath solution contained (in mM) 2.54 Ca²⁺, 159 Cl⁻, 149.2 Na⁺, 4.74 K⁺, 5.5 glucose, and 5 HEPES (300 mmol/kg osmolality, with final pH adjusted to 7.35 with NaOH) for whole cell recordings. All drugs added to extracellular Ringer solution were allowed to equilibrate with the cells for 2 min before data were recorded.

The amphipathic compounds LPC, LPA, and choline (Sigma-Aldrich) were used to increase lateral tension and deformation of the lipid bilayer (Fig. 2). With its large polar head group and single fatty acid tail, LPC will exert more tension in the polar region of the lipid bilayer. This change in membrane characteristics is proposed to alter ion channel conformation, such that LPC may cause ion channels to assume more of a barrel-shaped conformation with a shorter pore (40). Conversely, LPA, like arachidonic acid (AA), lacks a large polar head group and, therefore, is likely to transfer tension to the hydrophobic region of the lipid bilayer, which has been proposed to cause ion channels to assume more of an hourglass shape (40). These conformational changes have been linked to mediating ion channel mechanosensitivity (40). Although LPA shares the same tail as LPC, LPA would more likely influence mechanosensitivity of two-pore domain channels, such as the TREK family (32), rather than L-type Ca²⁺ channels; however, LPA also has been shown to affect intracellular Ca²⁺ stores (29, 30). Choline, entirely missing a fatty acid tail, would reside outside the hydrophobic region.

Data analysis. Values are means ± SE. Raw values from the same cells before and after addition of drug or control solution were evaluated by Student’s t-test (two-tailed). P < 0.05 was considered significant. Current-voltage curves plotted peak inward current vs. voltage steps. Data were normalized using the following formula: \(I/_{\text{norm}} = I_{\text{I}}/I_{\text{max}}\), where \(I_{\text{I}}\) is the normalized peak inward current at a particular voltage sweep, \(I_{\text{max}}\) is the maximum peak inward current from the set of traces (usually the peak inward current at 0 mV). Steady-state activation and inactivation curves were fit with the Boltzmann equation:

\[
\frac{I_{\text{MAX}}}{I} = \frac{1}{1 + \exp(V_{1/2} - V/k)},
\]

where \(V_{1/2}\) is the voltage at half-maximal activation or inactivation.

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Fig. 1. Time line showing 7-step experimental protocol of mechanical stimulus and application of amphipathic compounds. LPC, lysophosphatidyl choline.

Fig. 2. Molecular space-fill models of LPC, lysophosphatidic acid (LPA), and choline. Charge and shape of amphipathic compounds direct location of incorporation into the lipid bilayer and induce a subsequent conformational change of proteins embedded in the bilayer.
RESULTS

Membrane shear stress by fluid perfusion increases Ca\(^{2+}\) current. Under control conditions, perfusion increased inward L-type Ca\(^{2+}\) current (33 ± 5\% from baseline to 1st perfusion, n = 6, P < 0.05; Fig. 3). This increase is consistent with our previously published data (9, 18, 24). The increase in Ca\(^{2+}\) current (peak Ca\(^{2+}\) current) dissipated over the 8 min after cessation of fluid perfusion, and at 8 min there was no difference between pre- and postperfusion periods (10 ± 11\% from baseline to the end of the 1st postperfusion period, n = 6, P > 0.05). There was no effect of LPC on Na\(^{+}\) current (data not shown).

Effect of LPC on reversibility of mechanical activation of L-type Ca\(^{2+}\) current. Previous work showed that, in HEK-293 cells transfected with Cav1.2 (24), mechanical activation of L-type Ca\(^{2+}\) current is reversible on cessation of the stimulus. Similarly, control experiments in human jejunal circular smooth muscle cells demonstrated that L-type Ca\(^{2+}\) current was reversible after each successive perfusion. However, after the addition of LPC, Ca\(^{2+}\) current increased to a new plateau after the mechanical stimulation (48 ± 12\% from baseline to 2nd postperfusion, n = 6, P < 0.05; Fig. 4). Although Ca\(^{2+}\) current returned to baseline after the control perfusion (14 ± 6\%, n = 6, P > 0.05, Fig. 4), it remained at the level of perfusion-induced current increase in the presence of LPC (−4 ± 6\% from 2nd perfusion to the end of postperfusion, n = 6, P > 0.05; Fig. 4) and did not return to baseline.

Neither LPA nor choline affects Ca\(^{2+}\) current increase with shear stress. In contrast to LPC, which has a polar head and a single fatty acid chain, choline has a polar head but no fatty acid tail, and LPA has a fatty acid tail but no polar head (Fig. 2). The effect of these drugs was, therefore, tested as controls.

Fig. 3. Shear stress increased L-type Ca\(^{2+}\) current in circular smooth muscle cells of human jejunum. A: representative whole cell Ca\(^{2+}\) (arrow) currents before, during, and after 2 consecutive rounds of shear stress induced by perfusion. Arrowhead shows faster Na\(^{+}\) current. Inset: visual identification of Na\(^{+}\) (−30 mV, I\(_{\text{FAST}}\)) and Ca\(^{2+}\) (0 mV, I\(_{\text{SLOW}}\)) currents by step voltage and kinetics. B: current-voltage plots of peak inward currents from representative cell in A. I\(_{\text{FAST}}\) peaked at −30 to −25 mV; I\(_{\text{SLOW}}\) peaked at −5 to 5 mV. C: normalized peak Ca\(^{2+}\) currents averaged from 6 cells. *P < 0.05 vs. baseline (B). P1, 1st perfusion; PP1, 1st postperfusion; P2, 2nd perfusion; PP2, 2nd postperfusion.
for LPC. Separate double-perfusion experiments were performed with LPA and choline. In both sets of experiments, similar increases in $\text{Ca}^{2+}$ current were noted with the first (control) perfusion: 26 ± 4% increase from baseline ($n = 6$, $P < 0.05$; Fig. 5A) in the cells treated with LPA and 20 ± 4% increase from baseline ($n = 6$, $P < 0.05$; Fig. 5B) in the cells treated with choline. Neither LPA nor choline produced changes in $\text{Ca}^{2+}$ current within 2 min of application: 0 ± 2% with LPA ($n = 6$, $P > 0.05$; Fig. 5A) and −3 ± 5% with choline ($n = 6$, $P > 0.05$; Fig. 5B). Furthermore, neither compound changed the magnitude of the expected $\text{Ca}^{2+}$ current increase with the second perfusion: 20 ± 3% with LPA ($n = 6$, $P < 0.05$; Fig. 5A) and 19 ± 2% with choline ($n = 6$, $P < 0.01$; Fig. 5B). Mechanical activation of $\text{Ca}^{2+}$ current was also reversible in the presence of LPA or choline: 5 ± 5% and 0 ± 7% increase compared with preperfusion current for LPA and choline, respectively ($n = 6$ each, $P > 0.05$; Fig. 5).

**LPC, but not LPA or choline, alters steady-state kinetics of L-type $\text{Ca}^{2+}$ channel.** To further identify whether the altered response to shear stress in the presence of LPC was secondary to a change in the gating of the channel, we tested whether LPC alters steady-state kinetics of the L-type $\text{Ca}^{2+}$ channel in these human jejunal circular smooth muscle cells. After adding LPC, we observed a change in the slope [−11 ± 2 (control) and −15 ± 2 (LPC), $P < 0.05$] but not $V_{1/2}$ [−49 ± 2 mV (control) and −50 ± 3 mV (LPC), $P > 0.05$] of the inactivation curves (Fig. 6A, $n = 12$). We did not observe a change in slope of the activation curve $[5.7 \pm 0.3$ (control) and $6.5 \pm 0.8$ (LPC), $P > 0.05$], but we did observe a positive (5-mV) shift in $V_{1/2}$ $[-22 \pm 4$ mV (control) and $-17 \pm 1$ mV (LPC), $P < 0.05$, $n = 12$]. The fit was tighter for the activation kinetics than for inactivation. The net result of these changes in steady-state activation and inactivation kinetics due to LPC was an increase in the L-type $\text{Ca}^{2+}$ window current consistent with the increase in current seen after mechanical activation of the channel. Neither LPA nor choline altered the slope or $V_{1/2}$ of activation or inactivation steady-state kinetics (Fig. 6, B and C).

**DISCUSSION**

The L-type $\text{Ca}^{2+}$ channel expressed in human jejunal circular layer myocytes is mechanosensitive (9, 24), demonstrating an increase in $\text{Ca}^{2+}$ entry induced by membrane shear stress. The main finding of the present study was that a change in the tension of the lipid bilayer by addition of LPC increased mechanosensitivity of the L-type $\text{Ca}^{2+}$ channel. LPC increased window current through altered steady-state activation and inactivation kinetics, providing a likely mechanism for the observed increase in mechanosensitivity of the L-type $\text{Ca}^{2+}$ channel in the presence of LPC. The effect of LPC on the $\text{Ca}^{2+}$ current was not seen with choline or LPA, compounds in the same class of molecules but with significant structural differences.
Gastrointestinal motility is controlled at several levels. Enteric nerves generate stereotypic motor patterns, receive input from extrinsic nerves, and regulate the function of smooth muscle and interstitial cells of Cajal. Interstitial cells of Cajal generate the electrical slow wave (19, 48), modulate neuronal input to smooth muscle (3, 47), transduce mechanical information (39, 43, 46, 49), and regulate the membrane potential of smooth muscle (10, 35). Smooth muscle is not only the final common pathway for contractility, but it can also respond directly to external mechanical stimuli by changes in function of its ion channels, including opening of L-type Ca\(^{2+}\)/H\(^{+}\) channels and, thereby, increase Ca\(^{2+}\)/H\(^{+}\) entry (18) into the cell and, thus, contractile strength. Although the cytoskeleton has been shown to affect other voltage-gated Ca\(^{2+}\) channels in cells from other tissues (13, 16, 45), previous work on the human jejunal L-type Ca\(^{2+}\) channel showed that disruption of the cytoskeleton had no effect on its mechanosensitivity, suggesting that force was not transduced to the channel through the cytoskeleton (39). By exploiting different physical conformations of amphipathic compounds to change membrane tension, the data in our study suggest that force can be transmitted to the channel from the bilayer.

Amphipathic compounds such as LPC and LPA can incorporate into the lipid bilayer of cells or independently form micelles (23, 33, 40). Together with their role as structural elements of the membrane, lysophospholipids also modulate the function of several membrane proteins, including ion channels. LPC, LPA, or AA modulates TREK channels (4, 21, 25, 33), LPC modulates T-type Ca\(^{2+}\) channels (55), LPC or sphingosine-1-phosphate modulates transient receptor potential channel type 5 (1, 50), LPC modulates Na\(^{+}\) channels (14), and LPA modulates a Cl\(^{-}\) channel (52). The effects of lysophospholipids on these ion channels are diverse. Direct and indirect effects on membrane proteins have been demonstrated through a change in the physical properties of the membrane (56). Furthermore, lysophospholipids can also activate intracellular signaling pathways. LPA is known to exert effects on several intracellular signaling pathways, most notably G protein-coupled receptors (27). LPC also activates several pathways, including a mitogen-activated protein kinase pathway (42) and...
G protein receptor-coupled pathways (12). The results of the present experiments showed that LPC had no effect on the size of the baseline Ca\(^{2+}\) current or on baseline channel kinetics, yet LPC had a specific effect on mechanosensitivity, which supports the notion that the effects of LPC are mediated through changes in membrane tension, rather than activation of intracellular signaling cascades or changes in the membrane proteins. Also, prior work has not demonstrated an impact of disruption of channel phosphorylation on mechanosensitivity in intestinal L-type Ca\(^{2+}\) channels (24), again arguing against activation of a G protein-coupled receptor as a pathway accounting for the effects of LPC seen in the present study.

The effect of LPC on the L-type Ca\(^{2+}\) channel does not appear to be a nonspecific interaction between the lipid bilayer and an ion channel, because there was no effect of addition of LPC on Na\(_a\),1.5, another mechanosensitive ion channel expressed in human gastrointestinal smooth muscle cells. Na\(_a\),1.5 mechanosensitivity has been shown previously to depend on an intact cytoskeleton. Application of shear stress on the α-subunit of the L-type Ca\(^{2+}\) channel expressed in human embryonic kidney cells is sufficient to reproduce mechanosensitivity (24), suggesting a direct interaction between the pore-forming subunit of the L-type Ca\(^{2+}\) channel and the membrane. The increase in Ca\(^{2+}\) current in response to mechanical stimulation was transient under control conditions. Within 8 min of cessation of shear stress to the membrane, the Ca\(^{2+}\) current returned to pre-shear stress levels, suggesting that the mechanism that allows transmission of force to the pore-forming subunit is readily reversible. The failure of the L-type Ca\(^{2+}\) current to return to pre-shear stress values after addition of LPC, but not after LPA or choline, suggests that the level of membrane tension is an important determinant of the sustained response to a stimulus. The mechanisms by which force from the lipid bilayer alters Ca\(^{2+}\) channel kinetics are not known. Potential reasons for this include a limitation of the ability of the L-type Ca\(^{2+}\) channel to undergo conformational changes once mechanoinactivated or a different energy requirement to change states as a result of the increased tension in the lipid bilayer.

Analysis of the activation/inactivation kinetics of the channel with LPC in the bath solution revealed changes in activation and inactivation of the L-type Ca\(^{2+}\) channel, leading to a larger window current than observed in normal extracellular fluid or in the presence of LPA or choline. This change was not seen unless the channel was mechanically activated. The change in the window current observed in the presence of LPC is of likely importance, because it not only reflects increased current at the baseline “window” voltages but, also, a rightward shift in the activation curve toward membrane potentials that are likely closer to the physiological resting membrane potential of intestinal myocytes.

LPC, a product of hydrolysis of oxidized phospholipids, is generated under physiological and pathophysiological conditions (6, 34, 51). Micromolar concentrations can be found in plasma (5). Production of LPC from membrane phosphatidylcholines is regulated tightly. The concentration of LPC in the plasma is dependent on paraoxonases (34) and lysoospholipases (28) and is altered in disease states such as ischemia (6, 36) and cancer (8, 54). The concentrations of LPC used in the present study are within the range that may occur in the human body. Given that a particular gastrointestinal smooth muscle cell is constantly either actively contracting or being deformed by neighboring contracting cells, the concentration of LPC may alter Ca\(^{2+}\) entry into gastrointestinal smooth muscle, thereby altering contractility.

In summary, the data presented in this report suggest that mechanosensitivity of the L-type Ca\(^{2+}\) channel in human gastrointestinal circular smooth muscle is modulated by LPC, likely through a change in lipid bilayer tension. This regulatory mechanism may have pathophysiological relevance.

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