The GPR55 agonist lysophosphatidylinositol acts as an intracellular messenger and bidirectionally modulates Ca\(^{2+}\)-activated large-conductance K\(^+\) channels in endothelial cells

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Abstract Lysophospholipids are known to serve as intracellular messengers affecting many physiological processes. Lysophosphatidylinositol (LPI), which is produced in endothelial cells, acts as an endogenous agonist of the orphan receptor, G protein-coupled receptor 55 (GPR55). Stimulation of GPR55 by LPI evokes an intracellular Ca\(^{2+}\) rise in several cell types including endothelial cells. In this study, we investigated additional direct, receptor-independent effects of LPI on endothelial large-conductance Ca\(^{2+}\) and voltage-gated potassium (BK\(_{Ca}\)) channels. Electrophysiological experiments in the inside-out configuration revealed that LPI directly affects the BK\(_{Ca}\) channel gating properties. This effect of LPI strictly depended on the presence of Ca\(^{2+}\) and was concentration-dependent, reversible, and dual in nature. The modulating effects of LPI on endothelial BK\(_{Ca}\) channels correlated with their initial open probability (Po): stimulation at low Po (<0.3) and inhibition at high Po levels (>0.3). In the whole-cell configuration, LPI in the pipette facilitated membrane hyperpolarization in response to low (0.1–2 \(\mu\)M) histamine concentrations. In contrast, LPI counteracted membrane hyperpolarization in response to supramaximal cell stimulation with histamine. These results highlight a novel receptor-independent and direct bidirectional modulation of BK\(_{Ca}\) channels by LPI on endothelial cells. We conclude that LPI via this mechanism serves as an important modulator of endothelial electrical responses to cell stimulation.

Keywords BK\(_{Ca}\) channel · Cytosolic free Ca\(^{2+}\) elevation · Endothelial cells · Hyperpolarization · Membrane potential · Lipid mediators · Lysophosphatidylinositol

Introduction

Lysophosphatidylinositol (LPI) belongs to the class of lysophospholipids and is generated by phosphatidylinositol hydrolysis via the action of the Ca\(^{2+}\)-dependent phospholipase A2 [2, 27] and Ca\(^{2+}\)-independent phospholipase A1 [27, 45]. LPI has been found to contribute to many physiological and pathophysiological processes including reproduction, angiogenesis, cell proliferation, apoptosis, inflammation, cardiovascular, and autoimmune diseases [6, 10, 25]. Because of the discovery that LPI serves as a putative endogenous agonist of the orphan receptor G protein-coupled receptor 55 (GPR55) [24, 32, 33, 42], which leads to mobilization of intracellular Ca\(^{2+}\), LPI recently received particular attention. Moreover, LPI was shown to stimulate non-selective cation channels and inhibit the Na\(^+\)/K\(^+\) ATPase [4] GPR55-independently pointing to LPI as a putative vascular mediator.

An increase in intracellular free Ca\(^{2+}\) concentration represents a hallmark in endothelial cell physiology. Subsequently to the rise in cytosolic Ca\(^{2+}\), Ca\(^{2+}\)-dependent K\(^+\) channels (K\(_{Ca}\)) become activated [8, 9, 20] that they play a pivotal role in the regulation of vascular tone. In vascular smooth muscle cells, stimulation of large-
conductance Ca²⁺ and voltage-gated potassium (BK_Ca) channels counteracts depolarization during myogenic tone development, thus, limiting voltage-dependent Ca²⁺ entry and counteracting vasoconstriction. In endothelial cells, the stimulation of K_Ca contributes to vasodilatation by membrane hyperpolarization that increases the driving force for Ca²⁺ entry through non-voltage-gated Ca²⁺ channels that subsequently yields Ca²⁺-dependent formation of vasodilators, such like nitric oxide [18, 19, 30] or endothelium-derived hyperpolarizing factors [5, 16].

So far, stimulatory effects of LPI on BK_Ca and intermediate conductance K_Ca channels were reported to depend on the ability of LPI to mobilize internal Ca²⁺ [12, 38, 43]. However, recently several endogenous phospholipids, such like phosphatidylinositol 4, 5-bisphosphate or sphingosine-1-phosphate, have been shown to activate BK_Ca [26, 40] as well as TRPC channels [14] directly. Thus, the actual effects of LPI on vascular BK_Ca channels ought to be investigated in order to gain the understanding of the physiological potential of this putative inter- and intravascular mediator.

Accordingly, in order to explore potential direct effects of LPI on endothelial BK_Ca channels that lack the regulatory β-subunit [34], the effects of LPI were explored in excised membrane patches from human endothelial cells. We report herein that LPI directly modifies BK_Ca channel activity in a dual manner by either potentiating or inhibiting native BK_Ca channels at lower and higher basal activity of the channel, respectively. Altogether, our data indicate that LPI, besides its activity on GPR55, also exhibits receptor-independent effects, thus, pointing to LPI as a versatile messenger in the vasculature.

Materials and methods

Cell culture

The human umbilical vein-derived endothelial cell line, EA.hy926 [11], at passage >45, was grown in DMEM containing 10% FCS and 1% HAT (5 mM hypoxanthine, 20 μM aminopterin, 0.8 mM thymidine) and were maintained in an incubator at 37°C in 5% CO₂ atmosphere. For experiments, cells were plated on glass coverslips.

Patch clamp recordings

Single-channel recordings were obtained from excised inside-out membrane patches in symmetrical solutions using the patch clamp technique. The pipettes were filled with (in millimolar) 140 KCl, 10 HEPES, 1 MgCl₂, 5 EGTA, 4,931 CaCl₂ with pH 7.2 by adding KOH (i.e., 10 μM free Ca²⁺), calculated by CaBuf from G. Droogmans, Leuven, Belgium; ftp://ftp.cc.kuleuven.ac.be/pub/droogmans/cabuf.zip). Cells were perfused with a bath solution containing (in millimolar) 140 NaCl, 5 KCl, 1.2 MgCl₂, 10 HEPES, 10 glucose, 2,4 CaCl₂. Following gigaseal formation, bath solution was switched to the following (in millimolar) 140 KCl, 10 HEPES, 1 MgCl₂, 5 EGTA, and a desired free Ca²⁺ concentration which was adjusted by adding different amounts of CaCl₂ calculated by the program CaBuf; pH was adjusted to 7.1 by adding KOH.

For whole-cell recordings, the pipette solution contained (in millimolar) 100 K-aspartate, 40 KCl, 1 MgCl₂, 10 HEPES, 5 EGTA, and a free [Ca²⁺] was adjusted to 100 nM by adding 1,924 CaCl₂ calculated by the program CaBuf. Recordings were performed in high Na⁺ solution stated above. Patch pipettes were pulled from glass capillaries using a Narishige puller (Narishige Co. Ltd, Tokyo, Japan), fire-polished, and had a resistance of 3–5 MΩ for whole-cell recordings and 5–7 MΩ for single-channel recordings. Currents were recorded using a patch clamp amplifier (EPC7, List Electronics, Darmstadt, Germany) at a bandwidth of 3 kHz. The signals obtained were low pass filtered at 1 kHz using an eight-pole Bessel filter (Frequency Devices) and digitized with a sample rate of 10 kHz using a Digidata 1200A A/D converter (Axon Instruments, Foster City, CA, USA). Data collection and analysis were performed using Clampex and Clampfit software of pClamp (V9.0, Axon Instruments). Single-channel activity was obtained from >20 s of continuous recording under each experimental condition. The mean open time (to) in multichannel patches was calculated from the relationship 

\[
to = \frac{N_p \times T}{\#o},
\]

where \#o is the number of openings during a given time period of observation (T) [40].

Statistics

Analysis of variance (ANOVA) was performed, and statistical significance was evaluated using Scheffé’s post hoc F test of the Prism 5 software for Windows (GraphPad Software, Avenida de la Playa, CA USA). Level of significance was defined as P<0.05.

Results

LPI directly affects the BK_Ca channel activity by shortening the channel closed time

To investigate whether LPI has a direct effect on BK_Ca channels, the action of LPI on BK_Ca channel activity was investigated in the inside-out configuration in the presence of physiological levels of Ca²⁺ and Mg²⁺, the two metal ligands that affect gating of BK_Ca channels. Figure 1a illustrates BK_Ca single-channel activities recorded in ex-
cised patches in the presence of 0.1 (top panel) and 0.3 μM free Ca$^{2+}$ (middle panel), as well as in the nominal absence of bath Ca$^{2+}$ (lower panel), prior (control) and after addition of 3 μM LPI to the cytosolic side of the patch. Channel openings are shown as upward deflections (c, closed; o, open). In the absence of Ca$^{2+}$, LPI has no effect on BK$_{Ca}$ channel activity (lower panels).

b Summary data for the effect of 3 μM LPI on BK$_{Ca}$ channel activity in the absence (0 mM Ca$^{2+}$; n=6) and presence of 0.1 (n=10) and 0.3 μM (n=21) free Ca$^{2+}$ in the bath. *p<0.05 vs. basal NPo in the absence of LPI.

Because Ca$^{2+}$ is required for the direct modulation of BK$_{Ca}$ channel activity by LPI. A

In order to characterize the effect of LPI in more detail, experiments with patches with only one active channel were performed. The existence of just one active BK$_{Ca}$ channel in the respective patches was determined by applying 10 μM Ca$^{2+}$ solution at the end of the experiments at a holding potential of +80 mV. From these experiments, we found that LPI increased the channel Po, while the single-channel amplitude remained unchanged (Fig. 3a). Moreover, the mean open time was moderately increased by LPI (Fig. 3b), while LPI strongly attenuated the mean closed time by ~77% reflecting an increase in opening frequency (Fig. 3c), thus indicating that the increase in Po by LPI is mainly due to a shortening of the closed time rather than a prolongation of the channel open time.

LPI dually modulates BK$_{Ca}$ channels depending on the basal channel activity. Because Ca$^{2+}$ is required for the stimulatory action of LPI, LPI may act via an increase in the apparent Ca$^{2+}$ sensitivity of the BK$_{Ca}$ channel. Thus, the effect of LPI on BK$_{Ca}$...
channel activity was studied in the presence of various Ca$^{2+}$ concentrations in the bath. Interestingly, depending on the bath Ca$^{2+}$ concentration, LPI exhibited opposite effects on the activity of the BK$_{Ca}$ channels. In particular, under conditions of low Ca$^{2+}$ in the bath solution (i.e., 0.1 and 0.3 μM free Ca$^{2+}$), which were associated with basal Po lower than 0.3, the addition of 3 μM LPI augmented BK$_{Ca}$ channel activity (Fig. 4a). In contrast, at high Ca$^{2+}$ concentrations in the bath (i.e., 1 and 10 μM) that yielded elevated basal BK$_{Ca}$ activity, LPI decreased the activity of the BK$_{Ca}$ channel (Fig. 4a). This inhibitory effect of LPI was observed both in multichannel patches (Fig. 4b) and patches containing one active channel (Fig. 4c), and occurred in a concentration-dependent manner. This concentration-dependent inhibitory effect of LPI was in a similar range than that for the activation properties of LPI (Fig. 4d). Dwell time analysis of experiments performed in single-channel patches revealed that upon LPI exposure, the mean open time slightly decreased by 16±10% (Fig. 4e), while the mean closed time strongly increased by 168±82% (Fig. 4f). The decrease in (N)Po upon LPI under conditions of high bath Ca$^{2+}$ is due to a pronounced increase in the mean closed time (a decrease in the frequency of openings) and a small decrease in the mean open time.

We hypothesized that these striking differences of the response of BK$_{Ca}$ channel activity to LPI is due to the variability of the basal Po values. If this is the case, the basal Po levels should correlate with the degree of changes
in BK$_{Ca}$ channel activities caused by LPI. Plotting NPo responses against the basal Po values of BK$_{Ca}$ channels in patches exposed to various bath Ca$^{2+}$ concentration at a holding potential of +40 mV (Fig. 4g) revealed a clear inverse correlation between these two parameters ($r^2=0.77$). This finding indicates that the overall effect of LPI indeed depends on the basal Po level and thus on the basal activities of BK$_{Ca}$ channels. Particularly, these data specify that for BK$_{Ca}$ channels with a basal Po higher than 0.3 LPI exhibits an inhibitory action, while at a lower basal activity of the channels, LPI yields a stimulation of BK$_{Ca}$ channel activity.

Because dual sensitivities to Ca$^{2+}$ and transmembrane voltage are key features of BK$_{Ca}$ channels, we next studied whether LPI affects the voltage-dependency of BK$_{Ca}$ channels. In single-channel recordings, the stepwise elevation of the holding potential from 20 to 80 mV considerably increased the BK$_{Ca}$ channel activity (Fig. 5a, left panels). This increase was further potentiated by 3 μM LPI at every potential tested (Fig. 5a, right panels). In terms of channel-opening characteristics at different voltages, LPI slightly affected the mean open time of the BK$_{Ca}$ channels (Fig. 5c), while the channel’s mean closed time was strongly reduced by LPI (Fig. 5d). These experiments suggest that voltage dependency of the BK$_{Ca}$ channels is barely affected by LPI.

Furthermore, weak voltage-dependent effect of LPI was observed in experiments depicted in Fig. 6 and was performed in the presence of a given Ca$^{2+}$ concentration of 10 μM. At negative voltages (i.e., −60 and −40 mV), when the channel activity was low (NPo=0.173 at −60 mV and 0.282 at −40 mV, Fig. 6a), LPI equally increased NPo values (2.7- and 2.5-fold, respectively) (Fig. 6b, c). However, at positive voltages (i.e., +40 and Vm=+60 mV) that were associated with high basal activity of the channel (NPo levels of 1.23 at +40 mV and 1.27 at +60 mV), LPI equally decreased the channel activity by ~29% and 28%, respectively (Fig. 6b, c). Altogether, these data indicate that LPI modifies BK$_{Ca}$ channel activity mainly through modulating Ca$^{2+}$ sensitivity of the BK$_{Ca}$ channels, and the effect of LPI strictly depends on the actual state of the activity of these channels.

**Intracellular LPI dually modifies endothelial electrical responses to histamine**

Because LPI dually modifies BK$_{Ca}$ channel activities in excised patches in a Ca$^{2+}$-dependent manner, we next explored the physiological relevance of these findings by testing the impact of physiologically reported LPI concentrations (i.e., from 0.1 to 10 μM; [44]) on endothelial electrical responses to histamine in conventional whole-cell recordings. Because in the endothelial cell type used for this study, histamine-induced hyperpolarization is partially underpinned by an activation of BK$_{Ca}$ channels [15], the effect of 0.1 or 1.0 μM LPI in the patch pipette on membrane hyperpolarization in response to various moderate histamine concentrations was tested. Histamine evoked membrane hyperpolarization of endothelial cells in a concentration-dependent manner (Fig. 7a, d). Under control conditions (no LPI in pipette) consecutive applications of 0.1, 0.5, and 2.0 μM histamine produced hyperpolarizing responses of 10.5±2.1 mV (n=19), 19.8±2.3 mV (n=16), and 24.8±4.4 (n=7) from the mean resting membrane potential of −33.8±1.7 mV (n=19) (Fig. 7d). In the presence of 0.1 or 1.0 μM LPI in the pipette, the resting membrane potential was not affected compared with control
conditions (−32.0±2.0 mV (n=12) and −32.4±1.6 (n=8), respectively). However, when 0.1 and 1.0 μM LPI was present in the pipette, the amplitude of cell hyperpolarization in response to low histamine concentrations increased (Fig. 7b–d). To provide a link between increased endothelial hyperpolarization to low histamine concentrations in the presence of intracellular LPI and stimulatory effect of LPI on BKCa channels, paired experiments were conducted in the presence and absence of iberiotoxin, a selective inhibitor of BKCa channels. In the absence of intrapipette LPI, iberiotoxin (100 nM) slightly but significantly (p<0.05) reduced the peak amplitude to 0.5 and 2 μM histamine from 23.5±3.3 to 20.9±2.7 (n=8) and from 28.5±3.0 to 25.4±3.3 mV (n=8), respectively. In the inhibitory effect of 3 μM LPI on BKCa channel activity. The patch was exposed to 1 μM Ca2+ at Vm=+40 mV. d Summary data of the concentration-dependency of the inhibitory effect of LPI on BKCa channel activity in patches exposed to 1 μM Ca2+ at Vm=+40 mV (n=6–21). *p<0.05 vs. control. The respective mean open time (e, n=6, *p<0.05) and the mean closed time (f, n=6, *p<0.05) of the BKCa channel prior (control) and after addition of 3 μM LPI (Vm=+40 to +60 mV). Data collected from patches exposed to 1 and 10 μM Ca2+ were pooled. g Correlation between the LPI-evoked alterations in BKCa channel activity (expressed as the ratio of NPo values in the presence and absence of 3 μM LPI) and basal Po values at Vm=+40 mV. Data points were obtained in the presence of 0.1, 0.3, 1, and 10 μM Ca2+.
presence of intrapipette LPI (1 μM), iberiotoxin (100 nM) slightly but significantly \( (p<0.05) \) reduced the peak amplitude to 0.5 and 2 μM histamine (Fig. 7e, f) from 29.4±2.8 to 24.9±2.9 mV \((n=7)\) and from 34.9±2.2 to 29.4±1.5 mV \((n=11)\), respectively. Remarkably, iberiotoxin failed to completely inhibit the stimulatory effect of LPI on endothelial hyperpolarization to 0.5 and 2 μM histamine (Fig. 7f), which might be due to direct LPI effect on ion channels other then BKCa. Nevertheless, these experiments clearly indicate that stimulatory effect of intracellular LPI on endothelial hyperpolarization to 0.5 and 2 μM histamine is partially attributed to stimulation of BKCa channels. These results confirm our findings in excised patches and indicate that LPI increases the

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**Fig. 5** Effect of LPI on voltage-sensitivity of BKCa channel. a The activity of BKCa channels prior (left) and after (right) bath application of 3 μM LPI. The patch was exposed to 1 μM Ca\(^{2+}\). Unitary currents were recorded at different membrane potentials as indicated. Upward deflections are the opening events of the channel (c, closed, o, open state). b Correlation of the effect of 3 μM LPI on the channels Po expressed as percent of control with the actual holding potential. The relationship between the mean open time (e) and mean closed time (d) of the channel with the actual holding potential in the absence (control) and presence of LPI (3 μM). Data presented are representative data and experiments were repeated four times with different patches that provided similar results.
sensitivity of endothelial cells to submicromolar concentrations of histamine by facilitating the Ca\(^{2+}\)-induced activation of BK\(_{\text{Ca}}\) channels.

To test the effect of LPI under maximal activation of endothelial cells, the cells were stimulated with 100 \(\mu\)M histamine in the absence or presence of 1 \(\mu\)M LPI in the pipette. Notably, 1 \(\mu\)M LPI in the pipette slightly reduced the endothelial cell peak hyperpolarization in response to supramaximal histamine concentration (i.e., 100 \(\mu\)M) from 34.9±1.5 mV \((n=24)\) to 31.4±1.4 mV \((n=40)\; (p=0.055)\) (Fig. 8a, b, d). To estimate the effect of intracellular LPI on the sustained component of endothelial hyperpolarization, comparison of the membrane potential recovery after the peak has been performed as described previously [3]. In the presence of intracellular LPI (1 \(\mu\)M), the membrane potential declined faster and hyperpolarization after 300 s was 71.7±4.7% of the peak level of −63.9±1.2 mV \((n=11)\). In contrast, in the absence of LPI in the patch pipette, the hyperpolarization to 100 \(\mu\)M histamine after 300 s was 90.1±5.6% \((p<0.05)\) of the peak level of −71±2.6 mV \((n=5)\). In the combined presence of external iberiotoxin (100 nM) and internal LPI (1 \(\mu\)M), the sustained component of endothelial hyperpolarization was further decreased as evidenced from further reduction of hyperpolarization after 300 s to 58.2±8.7% \((p<0.05)\) of the peak level of −60.1±3.6 mV. These data support our findings presented in isolated patches and indicate that LPI indeed exhibits inhibitory properties on BK\(_{\text{Ca}}\) channel activity.
under conditions of excessive endothelial cell stimulation, thus, approving the concept of the dual modulator function of LPI in the vascular wall.

Discussion

Like in many other cell types, in endothelial cells, LPI triggers an increase of the intracellular Ca$^{2+}$ concentration by either intracellular Ca$^{2+}$ mobilization and/or the activation of Ca$^{2+}$ influx that subsequently yields activation of BK$_{Ca}$ channels [1, 21, 37, 38]. Very recently, these effects could be attributed to the binding of LPI to the orphan receptor GPR55 endogenously expressed in endothelial cells [42] and in HEK293 cells artificially expressing GPR55 [22–24]. Moreover, stimulation of endothelial cells with LPI was shown to be accompanied by an activation of BK$_{Ca}$ channels that was thought to be a consequence of the LPI-elicited Ca$^{2+}$ signals [4]. In addition, LPI was shown to affect directly the activity of ion channels, including members of the transient receptor potential channel family [14, 41], endothelial non-selective cation channels [4], and the two-pore domain mechano-gated TREK-1 and TRAAK K$^{+}$ channels [31]. However, so far, no evidence has been provided for a direct G protein-coupled receptor-independent modulation of BK$_{Ca}$ channels by LPI.

The data presented herein demonstrate that LPI, in physiological relevant concentrations [10, 44], dually modifies endothelial BK$_{Ca}$ channel activity in isolated inside-out patches as well as in current clamp experiments. Although the presence of Ca$^{2+}$ was a prerequisite for the modulator effect of LPI on BK$_{Ca}$ channels, the
mode of the LPI-induced modulation of BKCa channel activity did not essentially depend on the actual Ca$^{2+}$ concentration or voltage but strictly depended on the basal activity of the channel yielding either pronounced augmentation at low basal activity or attenuation if the basal BKCa channel activity was high. A change from LPI-mediated channel activation (increase in NPo) to channel inhibition occurs at a Po value of approximately 0.3. Because endothelial BKCa channels lack the regulatory β-subunit [34], the experiments using excised membrane patches suggest that LPI exhibits its effects by a direct interaction with the pore-forming component of these ion channels. While our findings in excised patches excludes the involvement of second messengers in the LPI-mediated modulation of BKCa channels, an immediate alteration of the proteolipid environment by LPI that might account for alterations of BKCa channel activity cannot be excluded. However, all effects of LPI on BKCa channels we have observed were sustained, concentration-dependent, and reversible. Importantly, LPI was used in low micromolar concentrations, thus, well below the LPI critical micellar concentration of ~75 μM [13], ensuring that the LPI actions on BKCa channels are caused by LPI monomers and are not due to nonspecific effects of LPI as a detergent. Furthermore, effective LPI concentrations are within the range found under normal (<0.1 μM) and pathological condition (~15 μM) in human tissue fluids [10, 44] and plasma [39].

The observation that both activation and inhibition of BKCa channel activity by LPI occurred in the same concentration range indicates that LPI has similar potency for both phenomena, though it remains unclear whether or not both effects correspond to distinct or identical interaction sites of LPI on the BKCa channel pore protein.
The dual effects of LPI on endothelial BK$_{Ca}$ channels were observed both in multiple channel patches and in patches containing only one active channel, indicating that alterations in the channel activity may occur due to changes in the channel's Po rather than changes in the number of active channels per patch. Although the latter possibility cannot be entirely excluded, our observation that the level of potentiation of BK$_{Ca}$ channel activity was quantitatively similar ($p=0.34$) in patches containing one and several active channels favors for the possibility that modulation of BK$_{Ca}$ channel activity occurs without changes in the number of active channels per patch. Moreover, LPI did not affect single-channel current amplitude or its conductance, suggesting that LPI does not alter BK$_{Ca}$ channel function by changing the entire conformation of the channel protein. Analysis of single-channel kinetics revealed that LPI acts primarily via alterations of the mean closed time, while the mean open time was only moderately affected, thus indicating that LPI modulates the activity of BK$_{Ca}$ channels mainly via a destabilization of the closed states.

The current study describes LPI as an effective stimulator as well as inhibitor of endothelial BK$_{Ca}$ channels. Considering that the action of endothelium-dependent vasodilators is mostly underpinned by a rise in the cytosolic Ca$^{2+}$ concentration and membrane potential fluctuations, the dual modulation of endothelial BK$_{Ca}$ channels by LPI might be of considerable physiological importance and may reflect a new mechanism of vascular function control by lysophospholipids.

This assumption is directly supported in experiments using the whole-cell recordings approach. In moderately stimulated cells that developed weak to modest hyperpolarization due to the increased cytosolic Ca$^{2+}$ concentration and membrane potential fluctuations, the dual modulation of endothelial BK$_{Ca}$ channels by LPI might be of considerable physiological importance and may reflect a new mechanism of vascular function control by lysophospholipids. Strikingly, several substances including ethanol [29] and the xenoestrogen tamoxifen [35] were reported to dually affect BK$_{Ca}$ channel activity. However, ethanol exerts its modulator effect on BK$_{Ca}$ channels only at concentrations found in the circulation after excessive alcohol consumption (50–100 mM), and the effect of tamoxifen is only of clinical relevancy as this compound is therapeutically used as a competitive antagonist of the estrogen receptor. Nevertheless, among these agents that have a complex action on BK$_{Ca}$ channels, LPI is unique as it serves as signaling molecule in the vasculature and can be found naturally in the vasculature/blood under physiological and pathophysiological conditions [10, 44].

Recently, LPI was shown to activate non-selective cation channels both when applied extracellularly or in excised inside-out membrane patches [4, 41]. Therefore, it appears reasonable that LPI may affect ion channels from the outer as well as the inner side of the plasma membrane. However, charged lipids are generally thought to act on the external side of the membrane affecting BK$_{Ca}$ channels [7]. Therefore, BK$_{Ca}$ channels may probably directly sense LPI from both intracellular and extracellular sides of the membrane. It is known that LPI levels increase almost threefold within seconds upon stimulation of endothelial cells with bradykinin [28]. Such an increase of the cellular LPI content is not specific for bradykinin but is a consequence of elevated cytosolic free Ca$^{2+}$ [2]. Considering the dual effects of LPI on BK$_{Ca}$ channel activity and electrical responses reported in this study, we suggested that intracellularly generated LPI should dually regulate endothelial electrical responses to endothelium-dependent vasodilators. Hence, LPI may serve as potent inter- and
intracellular signaling molecule modulating BK$_{Ca}$ channels in the vasculature.

In conclusion, we provide new information regarding the effects of LPI on endothelial K$^+$ channels. We demonstrate a direct dual action of LPI on endothelial BK$_{Ca}$ channel gating that does not require other cytosolic factors but depend on a cytosolic Ca$^{2+}$ elevation. These results describe a novel mechanism of the action of LPI and point to LPI as a potential second messenger in endothelial cells. LPI functions as a direct receptor-independent dual modulator of BK$_{Ca}$ channels in endothelial cells that impacts electrical responses to agonists and, thus, may affect Ca$^{2+}$ entry via store-operated and agonist-induced Ca$^{2+}$ entry pathways [17]. This signaling of LPI might allow a fine tuning of Ca$^{2+}$ sensitive processes within vascular cells and hence essentially contribute to the control of adaptive blood flows in various organs and tissues.

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References

1. Begg M, Mo FM, Offertaler L, Batkai S, Pacher P, Razdan RK, Lovinger DM, Kunos G (2003) G protein-coupled endothelial receptor for atypical cannabinoid ligands modulates a Ca$^{2+}$-dependent K$^+$ current. J Biol Chem 278:46188–46194
2. Billah MM, Lapetina EG (1982) Formation of lysophosphatidylinositol in platelets stimulated with thrombin or ionophore A23187. J Biol Chem 257:5196–5200
3. Bondarenko A, Sagach V (2006) Na$^{-}$/K$^{+}$-ATPase is involved in the sustained ACh-induced hyperpolarization of endothelial cells from rat aorta. Br J Pharmacol 149:958–965
4. Bondarenko A, Waldeck-Weimer M, Naghdi S, Poteser M, Malli R, Graier WF (2010) GPR55-dependent and -independent ion signaling in response to lysophosphatidylinositol in endothelial cells. Br J Pharmacol 161:308–320
5. Chen GF, Suzuki H (1990) Calcium dependency of the endothelium-dependent hyperpolarization in smooth muscle cells of the rabbit carotid artery. J Physiol Lond 421:521–534
6. Choi JW, Lee CW, Chun J (2008) Biological roles of lysophospholipid receptors revealed by genetic null mice: an update. Biochim Biophys Acta 1781:531–539
7. Clarke AL, Petrou S, Walsh JV, Singer JJ (2003) Site of action of fatty acids and other charged lipids on BK$_{Ca}$ channels from arterial smooth muscle cells. Am J Physiol 284:C607–C619, Cell Physiol 8. Colden-Stanfield M, Schilling WP, Possani LD, Kunze DL (1990) Bradykinin-induced potassium current in cultured bovine aortic endothelial cells. J Membr Biol 116:227–238
9. Colden-Stanfield M, Schilling WP, Ritchie AK, Eskin SG, Navarro LT, Kunze DL (1987) Bradykinin-induced increases in cytosolic calcium and ionic currents in cultured bovine aortic endothelial cells. Circ Res 61:632–640
10. Corda D, Iurisci C, Berrie CP (2002) Biological activities and metabolism of the lysophosphoinositides and glycerophosphoinositols. Biochim Biophys Acta 1582:52–69
11. Edgell CJ, McDonald CC, Graham JB (1983) Permanent cell line expressing human factor VIII-related antigen established by hybridization. Proc Natl Acad Sci USA 80:3734–3737
12. Erdogan A, Schaefer MB, Kuhlmann CR, Most A, Hartmann M, Mayer K, Renner FC, Schaefer C, Abdallah Y, Hoelschermann H, Schaefer CA (2007) Activation of Ca$^{2+}$-activated potassium channels is involved in lysophosphatidylcholine-induced monocyte adhesion to endothelial cells. Atherosclerosis 190:100–105
13. Falasca M, Corda D (1994) Elevated levels and mitogenic activity of lysophosphatidylinositol in k-ras-transformed epithelial cells. Eur J Biochem 221:383–389
14. Flemming PK, Dedman AM, Xu SZ, Li J, Zeng F, Naylor J, Benham CD, Bateson AN, Muraki K, Beech DJ (2006) Sensing of lysophospholipids by TRPC5 calcium channel. J Biol Chem 281:4977–4982
15. Frieden M, Graier WF (2000) Subplasmalemmal ryanodine-sensitive Ca$^{2+}$ release contributes to Ca$^{2+}$-dependent K$^+$ channel activation in a human umbilical vein endothelial cell line. J Physiol 524:715–724
16. Fukao M, Hattori Y, Kanno M, Sakuma I, Kitabatake A (1997) Sources of Ca$^{2+}$ in relation to generation of acetycholine-induced endothelium-dependent hyperpolarization in rat mesenteric artery. Br J Pharmacol 120:1328–1334
17. Girardin NC, Antigny F, Frieden M (2010) Electrophysiological characterization of store-operated and agonist-induced Ca$^{2+}$ entry pathways in endothelial cells. Pflugers Arch 460:109–120
18. Graier WF, Groschner K, Schmidt K, Kukovetz WR (1992) Increases in endothelial cyclic AMP levels amplify agonist-induced formation of endothelium-derived relaxing factor (EDRF). Biochem J 288:345–349
19. Graier WF, Kukovetz WR, Groschner K (1993) Cyclic AMP enhances agonist-induced Ca$^{2+}$ entry into endothelial cells by activation of potassium channels and membrane hyperpolarization. Biochem J 291:263–267
20. Groschner K, Graier WF, Kukovetz WR (1992) Activation of a small-conductance Ca$^{2+}$-dependent K$^+$ channel contributes to bradykinin-induced stimulation of nitric oxide synthesis in pig aortic endothelial cells. Biochim Biophys Acta 1137:162–170
21. Hasegawa Y, Erickson JR, Goddard GJ, Yu S, Liu S, Cheng KW, Eder A, Bandoh K, Aoki J, Jarosz R, Schrier AD, Lynch KR, Mills GB, Fang X (2003) Identification of a phosphothionate analogue of lysophosphatidic acid (LPA) as a selective agonist of the LPA3 receptor. J Biol Chem 278:11962–11969
22. Henstridge CM, Balenga N, Ford LA, Ross RA, Waldoher M, Irving AJ (2008) The GPR55 ligand 1-[(alpha)-lysophosphatidylinositol promotes RhoA-dependent Ca$^{2+}$ signaling and NFAT activation. FASEB J 23:183–193
23. Henstridge CM, Balenga NA, Schröder R, Kargl JK, Platzw, Martini L, Arthur S, Penman J, Whistler JL, Kostenis E, Waldoher M, Irving AJ (2010) GPR55 ligands promote receptor coupling to multiple signalling pathways. Br J Pharmacol 160:604–614
24. Kapur A, Zhao P, Sharir H, Bai Y, Caron MG, Barak LS, Abood ME (2009) Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. J Biol Chem 284:29817–29827
25. Karlner JS (2002) Lysophospholipids and the cardiovascular system. Biochim Biophys Acta 1582:216–221
26. Kim MY, Liang GH, Kim JA, Kim YJ, Oh S, Suh SH (2006) Sphingosine-1-phosphate activates BKCa channels independently of G protein-coupled receptor in human endothelial cells. Am J Physiol Cell Physiol 290:C1000–C1008
27. Kobayashi T, Kishimoto M, Okuyama H (1996) Phospholipases involved in lysophosphatidylinositol metabolism in rat brain. J Lipid Mediat Cell Signal 14:33–37
28. Lambert TL, Kent RS, Whorton AR (1986) Bradykinin stimulation of inositol polyphosphate production in porcine aortic endothelial cells. J Biol Chem 261:15288–15293
29. Liu J, Vaithianathan T, Manivannan K, Parrill A, Dopico AM (2008) Ethanol modulates BKCa channels by acting as an adjuvant of calcium. Mol Pharmacol 74:628–640
30. Luckhoff A, Busse R (1990) Calcium influx into endothelial cells and formation of endothelium-derived relaxing factor is controlled by the membrane potential. Pflugers Arch 416:305–411
31. Maingret F, Patel AJ, Lesage F, Lazdunski M, Honoré E (2000) Lysophospholipids open the two-pore domain mechanogated K+ channels TREK-1 and TRAAK. J Biol Chem 275:10128–10133
32. Oka S, Nakajima K, Yamashita A, Kishimoto S, Sugiura T (2007) Identification of GPR55 as a lysophosphatidylinositol receptor. Biochem Biophys Res Commun 362:928–934
33. Oka S, Toshida T, Maruyama K, Nakajima K, Yamashita A, Sugiuira T (2008) 2-Arachidonoyl-sn-glycero-3-phosphoinositol: a possible natural ligand for GPR55. J Biochem 145:13–20
34. Papassotiriou J, Kohler R, Prener J, Krause H, Akbar M, Eggermont J, Paul M, Distler A, Nilius B, Hoyer J (2000) Endothelial K+ channel lacks the Ca2+ sensitivity-regulating beta subunit. FASEB J 14:885–894
35. Pérez GJ (2005) Dual effect of tamoxifen on arterial KCa channels does not depend on the presence of the beta1 subunit. J Biol Chem 280:21739–21747
36. Petrou S, Ordway RW, Kirber MT, Dopico AM, Hamilton JA, Walsh JV, Singer JJ (1995) Direct effects of fatty acids and other charged lipids on ion channel activity in smooth muscle cells. Prostaglandins Leukot Essent Fatty Acids 52:173–178
37. Repp H, Birringer J, Koschinski A, Dreyer F (2001) Activation of a Ca2+-dependent K+ current in mouse fibroblasts by sphingosine-1-phosphate involves the protein tyrosine kinase c-Src. Naunyn Schmiedebergs Arch Pharmacol 363:295–301
38. Schilling T, Repp H, Richter H, Koschinski A, Heinemann U, Dreyer E (2002) Lysophospholipids induce membrane hyperpolarization in microglia by activation of IKCa1 Ca2+-dependent K+ channels. Neuroscience 109:827–835
39. Shen Z, Wu M, Elson P, Kennedy AW, Belinson J, Casey G, Xu Y (2001) Fatty acid composition of lysophosphatidic acid and lysophosphatidylinositol in plasma from patients with ovarian cancer and other gynecological diseases. Gynecol Oncol 83:25–30
40. Vaithianathan T, Bukiya A, Liu J, Liu P, Asuncion-Chin M, Fan Z, Dopico A (2008) Direct regulation of BK channels by phosphatidylinositol 4,5-bisphosphate as a novel signaling pathway. J Gen Physiol 132:13–28
41. Vanden Abeele F, Zholos A, Bidaux G, Shuba Y, Thebault S, Beck B, Flourakis M, Panchin Y, Skryma R, Prevarskaya N (2006) Ca2+-independent phospholipase A2-dependent gating of TRPM8 by lysophospholipids. J Biol Chem 281:40174–40182
42. Waldeck-Weiermair M, Zoratti C, Osibow K, Balenga N, Goessnitzer E, Waldhoer M, Malli R, Graier WF (2008) Integrin clustering enables anandamide-induced Ca2+ signaling in endothelial cells via GPR55 by protection against CB1-receptor-triggered repression. J Cell Sci 121:1704–1717
43. Wolfram Kuhlmann CR, Wiebke Lüdders D, Schaefer CA, Kerstin Most A, Backenköhler U, Neumann T, Tillmanns H, Erdogan A (2004) Lysophosphatidylcholine-induced modulation of Ca2+-activated K+ channels contributes to ROS-dependent proliferation of cultured human endothelial cells. J Mol Cell Cardiol 36:675–682
44. Xiao YJ, Schwartz B, Washington M, Kennedy A, Webster K, Belinson J, Xu Y (2001) Electrospray ionization mass spectrometry analysis of lysophospholipids in human ascitic fluids: comparison of the lysophospholipid contents in malignant vs nonmalignant ascitic fluids. Anal Biochem 290:302–313
45. Yamashita A, Kumasawa T, Koga H, Suzuki N, Oka S, Sugiuira T (2010) Generation of lysophosphatidylinositol by DDHD domain containing 1 (DDHD1): possible involvement of phospholipase D/phosphatic acid in the activation of DDHD1. Biochim Biophys Acta 1801:711–720