

G protein-activated K^+ channels: a reporter for rapid activation of G proteins by lysophosphatidic acid in *Xenopus* oocytes

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Abstract Threshold concentrations of lysophosphatidic acid (LPA) or acetylcholine (ACh) induce pertussis toxin (PTX)-sensitive rapid desensitization of responses to LPA in *Xenopus* oocytes. To demonstrate that threshold [LPA] rapidly activates Gi/o proteins, we used the G protein-activated K^+ channel (GIRK) as a reporter. Low [LPA] induced I_{K^+} in <3 s of the agonist addition with little or no activation of chloride current. Depletion of G α /G α 1 each decreased the LPA-induced I_{K^+} by approximately 40–50%, while PTX completely abolished it. This is the first direct evidence showing the activation of GIRK by LPA, and the involvement of G proteins of the Go family in rapid desensitization of LPA responses.
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Key words: Rapid desensitization; G protein-activated potassium channel; Lysophosphatidic acid receptor; Go/i protein

1. Introduction

Stimulation of G protein-coupled receptors (GPCRs) in *Xenopus laevis* oocytes leads to the activation of phosphoinositide-phospholipase C and inositol-trisphosphate-dependent calcium mobilization (PI-PLC-InsP₃-Ca²⁺) pathway. In *Xenopus* oocytes the physiological output is gating of calcium-sensitive chloride channels [1,2]. We have previously reported rapid desensitization of responses to thyrotropin-releasing hormone (TRH) receptor expressed in *Xenopus* oocytes, in which brief stimulation with threshold TRH concentrations caused significant desensitization of responses to a subsequent challenge with high agonist concentration [3]. Recently, we have reported that the Go family of G proteins mediates rapid homologous desensitization of responses to the stimulation of the endogenous receptor to lysophosphatidic acid (LPA) [4]. Based on previous reports [5–7] and on our recent publication, we proposed that different G protein families play different roles in activation and regulation of GPCR-induced responses. High concentrations of TRH or LPA induce calcium-activated chloride current, mainly via the activation of G pro-

teins of the Gq family [8–10], while threshold concentrations of LPA activate mainly Go proteins to desensitize the calcium mobilization pathway [4]. We did not, however, demonstrate directly that threshold LPA activates the Go proteins.

G protein-activated K^+ (GIRK) channels (or Kir3) mediate GPCR-induced responses in atrium and brain. The GIRK family includes five genes, each encoding a separate subunit, while a functional channel is composed of four subunits [11–14]. Heterotetrameric GIRK1/GIRK2, GIRK1/GIRK3 and GIRK2/GIRK3 channels exist in the brain and GIRK1/GIRK4 in the heart. G α subunits play an important role in determining the specificity of GPCR–GIRK signaling. In cardiac and neural cells only GPCRs coupled to pertussis toxin (PTX)-sensitive G α i/o activate GIRK [13,15,16]. Hence, agonist-dependent activation of GIRK reflects activation of Gi/o proteins. Here, by expression of the rodent heterotetrameric GIRK1/GIRK2, we demonstrate that threshold [LPA] rapidly activates the Go family of G proteins.

2. Materials and methods

2.1. Handling of oocytes

Stage V oocytes were isolated and maintained as previously described [17]. Oocytes were injected with rodent GIRK1/GIRK2 mRNA (5–25 pg/oocyte) and antisense deoxyoligonucleotides (ASODNs) (three phosphorothioate residues each at the 3' and 5' ends, 50 ng/oocyte), where appropriate. After injection, oocytes were maintained for an additional 48–72 h in NDE96 [18]. GIRK channels were measured in high- K^+ (HK) solution (ND96 changed to 24 or 96 mM KCl, balanced by appropriate changes in NaCl concentration) as described [11,12].

2.2. Functional assay in oocytes

Electrophysiological responses to agonists were recorded using two-electrode voltage clamp as described [19]. Current–voltage (I – V) characteristics were recorded by using voltage ramps (between –120 mV and 60 mV). The mean amplitude of the control group in each treatment was set as 100%, and the effect of each treatment was calculated as a percentage of the control response.

ASODN sequences, reverse transcription polymerase chain reaction (RT-PCR), quantitative real-time PCR and Western analysis were described previously [4].

2.3. Materials

ASODNs were synthesized commercially. LPA and acetylcholine (ACh) were from Sigma (Israel). Superscript enzyme was from Gibco BRL (Israel). RNasin was from Promega (Israel). SYBR was from Roche (Germany). Taq DNA polymerase was from Tal-Ron (Israel). PTX (A protomer) was from List Biological (USA). All other chemicals were of analytical grade.

2.4. Statistical analysis

All experiments were performed several times on a number of oo-

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Abbreviations: ASODN, antisense oligodeoxynucleotide; LPA, lysophosphatidic acid; GIRK, G protein-activated K^+ channel; TRH, thyrotropin-releasing hormone; PTX, pertussis toxin; GPCR, G protein-coupled receptor; PCR, polymerase chain reaction

cytes, obtained from a number of donor *Xenopus* females. Results are presented as mean \pm S.E.M.

3. Results

Xenopus oocytes respond to a challenge with LPA by a rapid transient chloride current [20,21]. Similarly to the previously described desensitization of the response to TRH [3], the response to LPA exhibits rapid homologous desensitization. Challenge with threshold [LPA] (5.0 nM) yielded very small responses. When oocytes were challenged again 30 s later with 0.5 μ M LPA, the resulting response was reduced by $69 \pm 6\%$ ($n = 10$). Similarly, in oocytes expressing the rodent M1 muscarinic receptor, 30 s challenge with 0.2 μ M ACh caused $42 \pm 9\%$ heterologous desensitization of the response to 0.5 μ M LPA ($n = 5$). Injection of the active protomer of PTX virtually abolished rapid homologous and heterologous desensitization (Fig. 1), strongly suggesting the involvement of the Gi/o family of G proteins in rapid desensitization. Our recent results were consistent with the LPA response being mediated mainly by the Gq family, while rapid homologous desensitization was mediated by the Go family of G proteins [4].

The results described above implied that short challenge of oocytes with threshold concentrations of LPA causes activation of Go proteins. To address this question, we expressed rodent GIRK1 (rat) GIRK2 (mouse) in oocytes. It has been shown that the expression of GIRK in oocytes leads to inward current upon elevation in extracellular K^+ concentration, which is further enhanced by the activation of M2 muscarinic receptors [22]. Muscarinic stimulation of GIRK current proceeds via the release of $\beta\gamma$ subunits from PTX-sensitive G proteins. In GIRK-expressing oocytes, changing the bath solution to 96 mM KCl caused a rapid increase in depolarizing current (Fig. 2, inset), displaying an I - V relationship with strong inward rectification typical for GIRK (Fig. 2). A challenge with 5 nM LPA caused an additional rapid increase in K^+ current. A similar experiment in oocytes challenged with 0.5 μ M LPA demonstrated initial fast gating of K^+ current, which then rapidly developed into a large chloride current (Fig. 3). This is confirmed by the changes in the I - V relationship: after the change of solution to HK, and during the first 2–3 s after the addition of LPA (L1), a typical inward-rectifying I - V curve is observed. However, approxi-

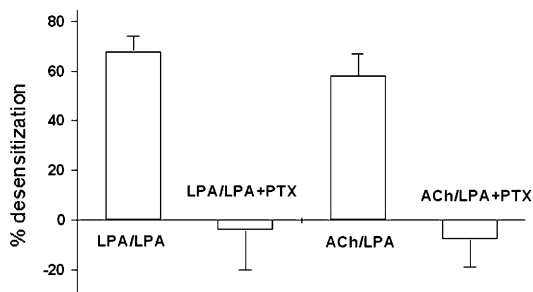


Fig. 1. PTX abolishes rapid homologous and heterologous desensitization of LPA response. Native or M1R-expressing oocytes were challenged with either LPA (5 nM) or ACh (0.2 μ M) and assayed 30 s later by exposure to 0.5 μ M of LPA. Where indicated, PTX (A protomer, 0.2 μ g/oocyte) was injected 90–120 min before assay. The results are presented as mean \pm S.E.M. % desensitization of the control responses to LPA.

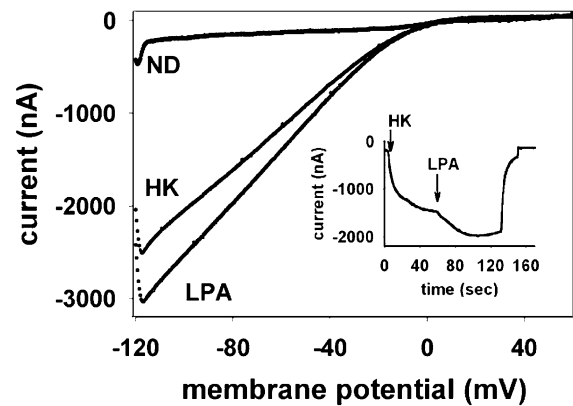


Fig. 2. Threshold [LPA] induces rapid gating of GIRK. Rodent GIRK was expressed in oocytes. I - V relationships in oocytes stimulated with 5 nM LPA are shown. Voltage ramps were applied before the switch to HK (ND), before the addition of LPA (HK), and at the plateau of the initial response to LPA (LPA). Inset shows a typical current response to medium change (from ND96 to 96 mM K^+ - HK) and to the subsequent stimulation with 5 nM LPA.

mately 7 s later, the I - V curves (L2, L3) show a clear outward current component at positive voltages, which also displays outward rectification, typical of Ca^{2+} -activated Cl^- channels in these cells.

To assess the role of Gi/o proteins in agonist activation of GIRK, we monitored the current amplitudes in oocytes challenged with 5 nM LPA and injected with ASODNs or the catalytic subunit of PTX. Anti-G α 1 or anti-G α o caused 41 ± 4 or $46 \pm 6\%$ inhibition of LPA-induced K^+ current, respectively (Fig. 4). Injection of PTX caused a complete abolition of K^+ currents (Fig. 4). Injection of anti-G α q had no effect on LPA-induced K^+ current (not shown). These results strongly suggested that LPA-induced K^+ currents were mediated to a large extent by G α o proteins and were compatible with early activation of GIRK conductance via the Gi/o family of G proteins at threshold [LPA]. A delayed gating of Ca^{2+} -activated chloride conductance via an endogenous G α q fam-

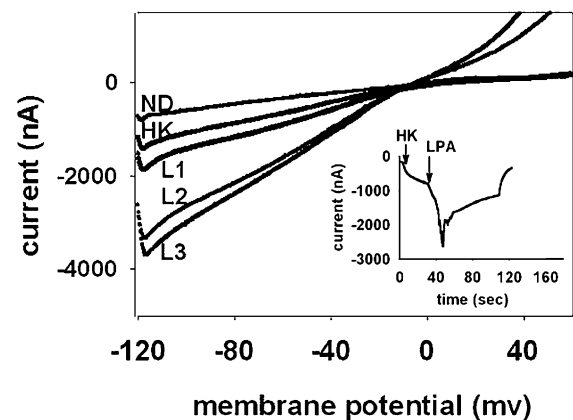


Fig. 3. High [LPA]-induced rapid gating of GIRK is followed by chloride current. I - V relationship in oocytes stimulated with 0.5 μ M LPA. Voltage ramps were applied before the switch to HK (ND), before the addition of LPA (HK), at the plateau of the initial response to LPA (L1) and at two points near the maximal response to LPA (L2, L3). The inset shows a typical current response to medium change (from ND96 to HK) and subsequent stimulation with 0.5 μ M LPA.

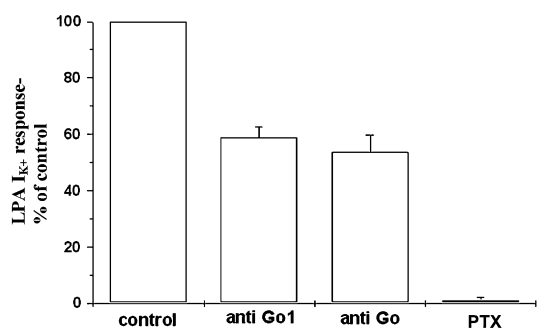


Fig. 4. The effect of ASODN-induced $G\alpha$ subunit depletion or PTX on the amplitude of LPA-evoked K^+ currents. I_{K^+} was measured as in the legend to Fig. 1 (at 5 nM LPA). Results are shown as % of control current amplitudes.

ily-mediated pathway, particularly at higher [LPA], was also observed, as has been shown previously [4].

4. Discussion

We first described rapid desensitization of expressed TRH receptors in *Xenopus* oocytes in 1995 [3]. In this desensitization paradigm, short exposure to threshold [TRH] (i.e. at very low receptor occupancy) caused significant desensitization to a subsequent challenge with high [TRH]. We hypothesized that during the exposure to threshold agonist concentration, an inhibitory pathway is initiated, which limits the subsequent response. Little, if any, activation of the PI-PLC-InsP₃-Ca²⁺ pathway was observed at threshold [TRH].

Native oocyte LPA receptors mediate large depolarizing Cl⁻ currents at optimal [LPA]. Recently, we demonstrated that threshold [LPA] causes extensive desensitization of response to high [LPA] [4]. This desensitization was mediated mainly by the Go family of G proteins, whereas high [LPA] also activates the Gq family of G proteins to produce a typical large Cl⁻ current response. It was demonstrated recently that oocytes express two subtypes of LPA receptors. Using specific ASODNs targeted against each receptor subtype transcript, we have shown that both subtypes equally contribute to rapid desensitization [4].

In the present report we also demonstrated that threshold concentrations of ACh caused heterologous desensitization of the response to LPA. The magnitude and the sensitivity to PTX of ACh/LPA desensitization were comparable to the previously reported [4] homologous desensitization. We postulated, therefore, that short exposure to threshold agonist concentration activates the Go family, without significant activation of the Gq family of G proteins. This report directly demonstrates the validity of this postulate.

In order to monitor rapid events occurring at low agonist concentration, we utilized LPA activation of the inward potassium rectifier channel, GIRK, as a reporter. It is now well established that the K⁺ conductance of GIRK expressed in *Xenopus* oocytes is rapidly activated by G protein $\beta\gamma$ subunits released from the Go/i family of G proteins. This is brought about by agonists of coexpressed Go/i-coupled receptors [11,23]. In fact, a number of receptors that can rapidly activate the Go/i family of G proteins have been shown to cause rapid activation of K⁺ current in cells that express the GIRK channel proteins [17,24]. Here we show that 5 nM LPA activates K⁺ conductance in oocytes expressing rodent GIRK.

This K⁺ current exhibits the predicted $I-V$ relationship with the characteristic inward rectification. It appears that low [LPA] preferentially activates this pathway. At higher [LPA], the activation of K⁺ currents is followed by a subsequent activation of Cl⁻ current, presumably via the PI-PLC-InsP₃-Ca²⁺ pathway in oocytes. This is directly observed as a time-dependent loss of inward rectification and the appearance of outward rectification.

We have recently shown for LPA-induced Cl⁻ currents that a minor part of the response is mediated by the Go family, whereas a major part is mediated by the Gq family of G proteins [4]. In the present study we have shown that depletion of $G\alpha o/G\alpha i$ each decreased the LPA-induced I_{K^+} by approximately 40–50%, while PTX completely abolished this current. The data on the LPA-induced response and its rapid desensitization [4], together with the present data on rapid activation of Go by low [LPA], suggest the following sequence of events: at threshold [LPA] (5 nM), the Go/i families of G proteins are rapidly activated. Co-expression of GIRK as a reporter allows monitoring of this event. The PI-PLC-InsP₃-Ca²⁺ pathway exhibits only weak activation and delayed kinetics (latency of approximately 16 s and <10% of response amplitude [4]). The activation of the Go family of G proteins initiates a rapid process of desensitization of a subsequent response to high [LPA] (0.5 μ M). Although our previous data on rapid desensitization of the LPA response strongly suggested that the Go family mediates this phenomenon, this is the first direct demonstration of threshold [LPA] rapidly activating Go/i proteins.

Our previous results [4] suggest that rapid homologous desensitization proceeds at least partially via events downstream of calcium mobilization. Kim described phosphorylation-linked rapid desensitization of muscarinic K⁺ current in atrial cardiomyocytes [25,26]. Vorobiov et al. [27] studied the agonist-induced desensitization of GIRK expressed in *Xenopus* oocytes, where the rapid component is lacking and only slow desensitization of GIRK can be studied. Indeed, their results demonstrated that there is an inherent slow desensitization of the GIRK current in oocytes when the channels were activated by high K⁺ ($t_{1/2} \approx 17$ min), which was further enhanced in the presence of the agonist, ACh ($t_{1/2} \approx 5$ –6 min) or the opiate agonist DAMGO ($t_{1/2} \approx 3$ min). These data suggest that rapid desensitization of the agonist-induced GIRK current is absent in oocytes. The logical conclusion of the study by Vorobiov et al. was that even the continuous presence of high agonist concentration does not rapidly affect the receptor, nor the Gi/Go proteins. Hence, rapid desensitization of the Gq-mediated chloride responses may be mediated at the Gq and/or steps distal to G protein activation.

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