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Inhibition of Mammalian Gq Protein Function by Local Anesthetics

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Background: **Local anesthetics have been shown to selectively inhibit functioning of *Xenopus laevis* Gq proteins. It is not known whether a similar interaction exists with mammalian G proteins. The goal of this study was to determine whether mammalian Gq protein is inhibited by local anesthetics.**

Methods: **In *Xenopus* oocytes, the authors replaced endogenous Gq protein with mouse Gq (expressed in Sf9 cells using baculovirus vectors). Cells endogenously expressing lysophosphatidic acid or recombinantly expressing muscarinic m3 receptors were injected with phosphorothioate DNA antisense (or sense as control) oligonucleotides against *Xenopus* Gq. Forty-eight hours later, oocytes were injected with purified mouse Gq (5×10^{-8} M) or solvent as control. Two hours later, the authors injected either lidocaine, its permanently charged analog QX314 (at IC_{50}, 50 nl), or solvent (KCl 150 mM) as control and measured Ca-activated Cl currents in response to lysophosphatidic acid or methylcholine (one tenth of EC_{50}).**

Results: **Injection of anti-Gq reduced the mean response size elicited by lysophosphatidic acid to 33 ± 7% of the corresponding control response. In contrast, responses were unchanged (131 ± 29% of control) in cells in addition injected with mouse Gq protein. Injection of mouse Gq protein "rescued" the inhibitory effect of intracellularly injected QX314: whereas QX314 was without effect on Gq-depleted oocytes, responses to lysophosphatidic acid after QX314 injection were inhibited to 44 ± 10% of control response in cells in addition injected with mouse Gq protein (5×10^{-8} M). Similar results were obtained for m3 signaling and intracellularly injected lidocaine.**

Conclusion: **Inhibition of Gq function by local anesthetics is not restricted to *Xenopus* G proteins. Therefore, Gq should be considered as one additional intracellular target site for local anesthetics, especially relevant for those effects not explainable by sodium channel blockade (*e.g.*, antiinflammatory effects).**

G-PROTEIN–coupled receptors (GPCRs) constitute one of the largest known protein families in mammals, including man. Nearly 2,000 GPCRs have been reported since bovine opsin was cloned in 1983 and the β-adrenergic receptor in 1986. Current estimates suggest that approximately 1% (about 1,000) of the genes present in a mammalian genome code for these types of receptors. They are of fundamental importance for intracellular and intercellular communication pathways. The majority of transmembrane signal transduction in response to stimuli as diverse as light, gustatory compounds, odorants, neurotransmitters, neuropeptides, hormones and glycoproteins is mediated by GPCRs. Particularly important for anesthesiologists are those GPCRs involved in transducing the functions of the autonomic nervous system, as well as receptors transducing the action of opiate narcotics, adenosine and related compounds, serotonin and related compounds, and α_2-adrenergic agonists. Also, many of the critical mediators of the inflammatory and hemostatic systems act through GPCRs. Examples include thrombin, thromboxane, platelet-activating factor (PAF), ADP, the interleukins, and compounds with less well-established physiology, such as the platelet activator and polymorphonuclear neutrophil (PMN) chemoattractant lysophosphatidic acid (LPA).

A number of GPCRs (*e.g.*, LPA, thromboxane A_2, trypsin, m1 and m3 muscarinic receptors) are inhibited by local anesthetics (LAs). Experiments in *Xenopus* oocytes, using antisense oligonucleotides directed against G-protein α subunits, demonstrated that each of these LA-sensitive GPCRs coupled (among other G proteins) to Gq. In contrast, angiotensin A_2 signaling was shown neither to be affected by LA nor to couple with Gq in this model.[1,2] This correlation between Gq coupling and LA sensitivity suggests the Gq protein as a target site for LAs. In other experiments, intracellularly injected QX314, a permanently charged and therefore membrane-impermeant lidocaine analog (on lysophosphatidic acid [LPA]), muscarinic m1 and m3 signaling), and lidocaine (on trypsin signaling) were rendered unable to inhibit signaling by prior selective depletion of Gq. In contrast, depletion of other G proteins did not affect LA action.[1,2]

If Gq is an intracellular target site for LAs, any receptor coupling to Gq would be expected to be affected by these compounds. These findings might explain some of the LA effects not primarily mediated by Na channel blockade, such as their antiinflammatory or antithrombotic actions.[3–5]

However, selective inhibition of Gq protein function has so far only been shown for the endogenous frog G

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protein. Although frog and mammalian G proteins are 90% homologous, the selectivity of LA is extremely high (*e.g.*, QX314 differentiates between Gq and G_{11}, which are 98% identical), and it therefore cannot be assumed that a similar interaction would exist between LA and mammalian Gq protein. If the interaction between LA and G proteins were restricted to amphibians, its relevance would obviously be decreased.

Therefore, in this study, we investigated whether the inhibitory effect of intracellularly injected LAs in frog Gq-depleted oocytes could be "rescued" by microinjection of mammalian Gq protein.

Materials and Methods

The studies were performed in *Xenopus* oocytes. These cells express endogenous LPA and trypsin receptors; other G-protein–coupled receptors can be expressed conveniently. Intracellular Ca release as a response to receptor stimulation is easily assessed as Ca-activated Cl currents, and the size of the cells makes intracellular injection straightforward. In addition, using oocytes allowed comparison with our previous results obtained in this model. The study protocol was approved by the Animal Research Committee at the University of Virginia (Charlottesville, Virginia). Oocyte harvesting, receptor expression, intracellular injections, drug administration, and electrophysiologic recording were performed as described previously.[1,2,6–8]

Oligonucleotide Injection

Phosphorothioate oligonucleotides were synthesized by the University of Virginia Research Facility (Charlottesville, Virginia). The antisense sequence is complementary to specific 20-base segments with less than 50% homology with other types of *X. laevis* Gα proteins.[9] Sense oligonucleotides were used as control. Uninjected oocytes (for experiments on the LPA receptor) or those injected 24 h prior with cRNA encoding the m3 receptor were injected with 50 nl sterile water containing 50 ng/cell antisense or sense oligonucleotides. Forty-eight hours after oligonucleotide injection, the cells were tested as described previously.

Drug Administration

Lysophosphatidic acid and methylcholine, used as agonists for the LPA or m3 muscarinic receptor, were diluted in Tyrode's solution to the required concentration and superfused (3 ml/min) over the oocyte for 10 s. The oocyte was positioned close to the inflow tubing so that complete exposure to test solutions was obtained in 4.8 ± 0.4 s (n = 20). Responses were quantified by measuring peak current and are reported as μA.

Intracellular Local Anesthetic Injections

For intracellular administration of QX314 or lidocaine, a third micropipette was inserted into the voltage-clamped oocyte. The micropipette was connected to an automated microinjector (Nanoject; Drummond Scientific, Broomall, PA). Under voltage clamp, 25 nl (approximately 5% of total oocyte volume) of a 300-mM KCl solution was injected for determination of the control response; in the treatment group, we injected 25 nl KCl solution containing various concentrations of QX314 or lidocaine. Injection was followed by superfusion with Tyrode's solution for 10 min, preventing an extracellular effect of any QX314 or lidocaine leaked from the puncture site or through the membrane. $I_{Cl(Ca)}$ was then induced by superfusion of LPA or methylcholine, as described previously. Control and treatment responses were obtained from different oocytes to prevent the effects of receptor desensitization from obscuring the results.

Expression and Purification of Mouse Gq Protein

The purification of Gq is based on the original method of Biddlecome *et al.*,[10] described in detail in Lindorfer *et al.*[11] Minor changes have been made to this protocol in an attempt to increase yields. Briefly, for the chromatographic separation of Gq using Ni^{2+}-NTA resin (Qiagen), the Q chromatography buffer was modified to contain the following: 20 mM Tris (pH 8.0), 150 mM NaCl, 1 mM $MgCl_2$, 0.5% (v/v) Genapol, 5 mM imidazole, 1 mM β-mercaptoethanol, 10 μM GDP, 17 μg/ml phenyl-methylsulfonyl fluoride (PMSF), and 2 μg/ml pepstatin, leupeptin, and aprotinin. Furthermore, during the washing of the Ni^{2+}-NTA column, the 1-M NaCl wash was removed, and the 0.2% cholate and 0.3% cholate–GTPγS washes were combined into one wash with 0.3% cholate and 3 μM GTPγS. Approximately 20–30 μg Gq was purified from a 20 g (wet wt.) Sf9 cell pellet. For experiments, 25 nl purified mouse Gq protein (final intracellular concentration 5×10^{-8} M) was dissolved in detergent (final intracellular concentration cholate 0.001%). Cholate in a final intracellular concentration of 0.001% was used as control.

Analysis

Results are reported as mean ± SD. Measurements of at least 22 oocytes were averaged to generate each data point. As variability between batches of oocytes is common, responses were at times normalized to control response. Statistically significant differences were assessed using one-way analysis of variance followed by Student-Newman-Keuls correction for multiple comparisons. $P < 0.05$ was considered significant. Concentration–response curves were fit to the following logistic function, derived from the Hill equation

$$y = y_{min} + (y_{max} - y_{min})\{1 - x^n/(x_{50}{}^n + x^n)\}$$

where y_{max} and y_{min} are the maximum and minimum response obtained, n is the Hill coefficient, and X_{50} is the

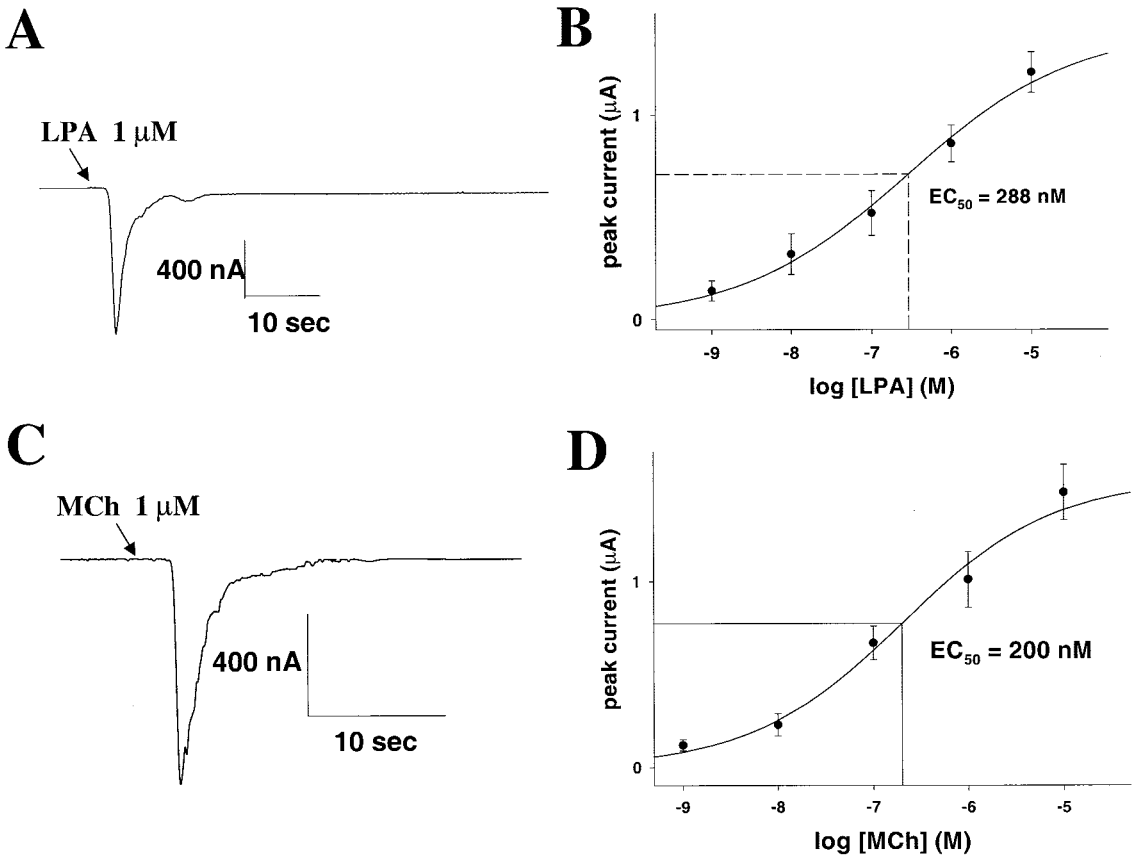

Fig. 1. (*A*) Example of an inward chloride current ($I_{Cl(Ca)}$) induced by 10 s administration of lysophosphatidate (LPA, 1 μM) in oocytes expressing endogenous LPA receptors (0.96 μA). (*B*) LPA evokes $I_{Cl(Ca)}$ in a concentration-dependent manner. Curve fitting using the Hill equation revealed a half-maximal effect concentration (EC_{50}) of 288 ± 30 nM. (*C*) Example trace of $I_{Cl(Ca)}$ induced by 10 s administration of methylcholine (MCh, 1 μM) in oocytes expressing muscarinic m3 receptors (0.87 μA). (*D*) Concentration–response relation for MCh-induced $I_{Cl(Ca)}$. Curve fitting using the Hill equation revealed an EC_{50} of 200 ± 70 nM.

half-maximal effect concentration (EC_{50} for agonist) or the half-maximal inhibitory effect concentration (IC_{50} for antagonist).

Materials

Molecular biology reagents were obtained from Promega (Madison, WI), and other chemicals were obtained from Sigma (St. Louis, MO). QX314 was a gift from Astra Pharmaceuticals, L.P. (Westborough, MA).

Results

Lysophosphatidic Acid Responses in Xenopus *Oocytes*

To provide baseline measurements and to confirm that our model functioned appropriately, we determined the concentration–response relation for LPA. LPA induced inward currents ($I_{Cl(Ca)}$) as described previously by our group[1,7,12] and others[13,14] (fig. 1A). As shown in figure 1B, the response to LPA was concentration dependent. EC_{50} was 288 ± 30 nM (n > 22 for each data point). Maximal responses of 1.2 ± 0.1 μA were obtained at an LPA concentration of 10 μM. Calculated E_{max} was 1.4 ± 0.2 μA, and the Hill coefficient was 0.42 ± 0.09. These findings are similar to those reported in our previous studies.[1,15]

Functional Expression of m3 Muscarinic Receptors in Xenopus *Oocytes*

Whereas uninjected oocytes were unresponsive to methylcholine, oocytes injected with m3 muscarinic receptor cRNA responded to application of methylcholine (10^{-5}–10^{-9} M) with a transient $I_{Cl(Ca)}$ (fig. 1C). We have shown previously that this response is mediated by m3 muscarinic receptors as it is inhibited by atropine and the selective m3 antagonist 4-diphenylacetoxy-N-methylpiperidine (4-DAMP).[16]

We determined the concentration–response relation for the m3 response. As shown in figure 1D, this response was also concentration dependent. EC_{50}, calculated from the Hill equation, was 200 ± 70 nM. Maximal responses of 1.5 ± 0.2 μA were obtained at an methylcholine concentration of 10 μM. Calculated E_{max} was 1.6 ± 0.2 μA. These findings also compare closely with data reported in our previous studies.[2,16–18]

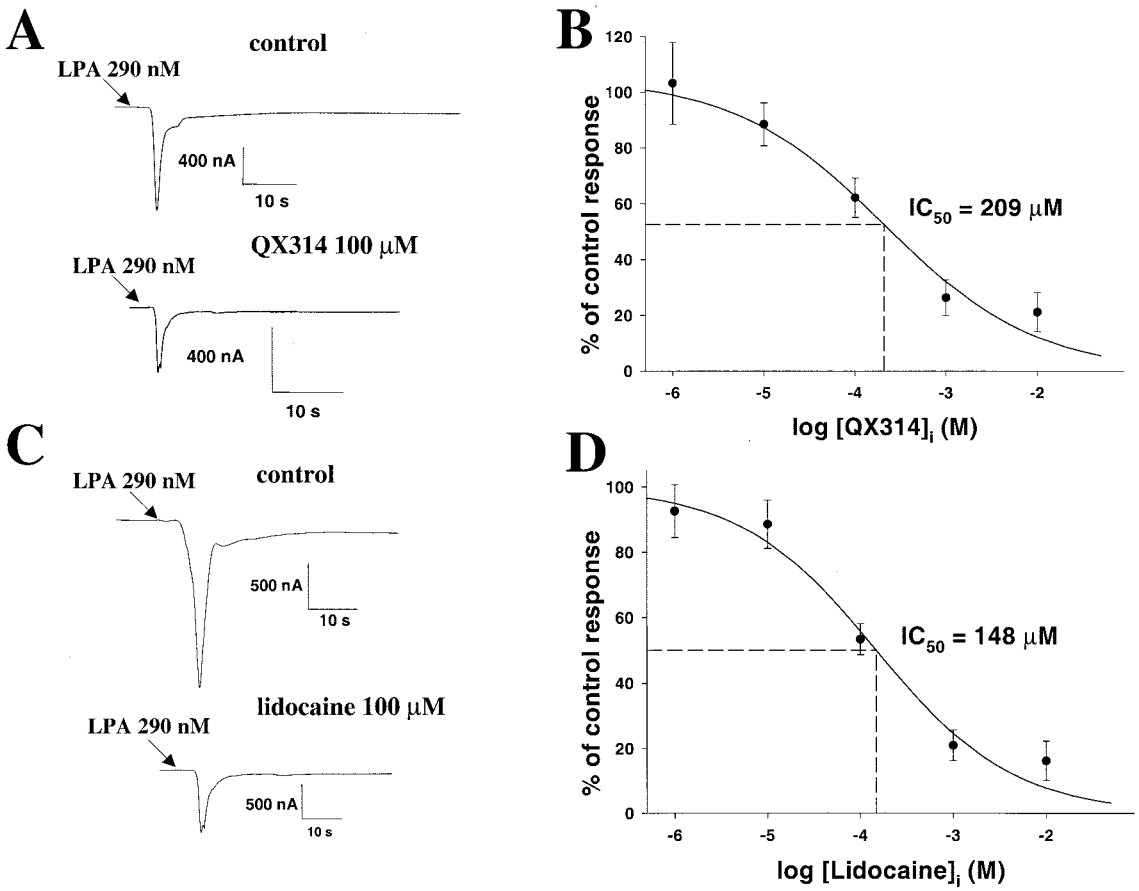

Fig. 2. (*A*) Example trace of LPA (at EC_{50} 290 nM)-induced $I_{Cl(Ca)}$, under control conditions (*top*, 1.05 μA) and 10 min after intracellular injection of QX314 (100 μM; *bottom*, 0.34 μA). (*B*) Intracellular injection of QX314 inhibits LPA (EC_{50})-induced $I_{Cl(Ca)}$ in a concentration-dependent manner. Curve fitting using the Hill equation revealed a half-maximal inhibitory concentration (IC_{50}) of 209 ± 164 μM. (*C*) Example trace of LPA (at EC_{50} 290 nM)-induced control response (*top*, 1.61 μA) and $I_{Cl(Ca)}$ after 10 min intracellular treatment with lidocaine on LPA response elicited by EC_{50} of LPA (*bottom*, 0.78 μA). (*D*) Intracellular lidocaine inhibited LPA responses with an IC_{50} of 148 ± 105 μM.

Concentration-dependent Inhibition of Lysophosphatidate Signaling by Intracellular QX314 or Lidocaine

We then studied the effect of intracellularly injected QX314 on LPA signaling. LPA-induced responses (at EC_{50}, 290 nM) were inhibited in a concentration-dependent manner after 10 min exposure to various concentrations of QX314. IC_{50} for intracellular QX314 was 209 ± 164 μM (figs. 2A and B). Maximal inhibition was obtained with QX314 10 mM; at this concentration, LPA responses were inhibited by 79%. The IC_{50} determined for intracellularly injected QX314 on LPA signaling (209 ± 164 μM) is similar to that obtained previously for m3 signaling (444 ± 226 μM),[2] which would be expected if the intracellular site of action is the same, namely the Gq protein.

Since we planned to use lidocaine in further experiments, we next studied the inhibitory potency of intracellularly injected lidocaine on responses elicited by administration of LPA at EC_{50} (290 nM). A sample trace of a LPA response after 10 min exposure to intracellular lidocaine (100 μM) is shown in figure 2C. Figure 2D illustrates the concentration–response relation for the effect of intracellular lidocaine. Curve fitting to the Hill equation revealed an IC_{50} of 148 ± 105 μM, which is very close to that determined previously for the intracellular inhibitory potency of lidocaine on trypsin signaling (445 ± 147 μM).[1] Maximal inhibition (84% of control response) occurred at an intracellular lidocaine concentration of 10 mM.

Effects of Mammalian Gq Protein

Depletion of endogenous frog Gq protein reduces response sizes and eliminates the inhibitory action of intracellular LA. To assure that this effect demonstrated in our previous article[1] also holds true for the different experimental conditions employed in the current study, we first determined whether oocytes injected with sense oligonucleotides (in KCl and detergent carrier) maintain a normal sensitivity to LAs. In the same set of experiments, we also verified that under the same conditions, anti-Gq–injected oocytes loose sensitivity to LA. As shown in figure 3, mean control response for oocytes injected with Gq sense, KCl, and detergent, elicited by

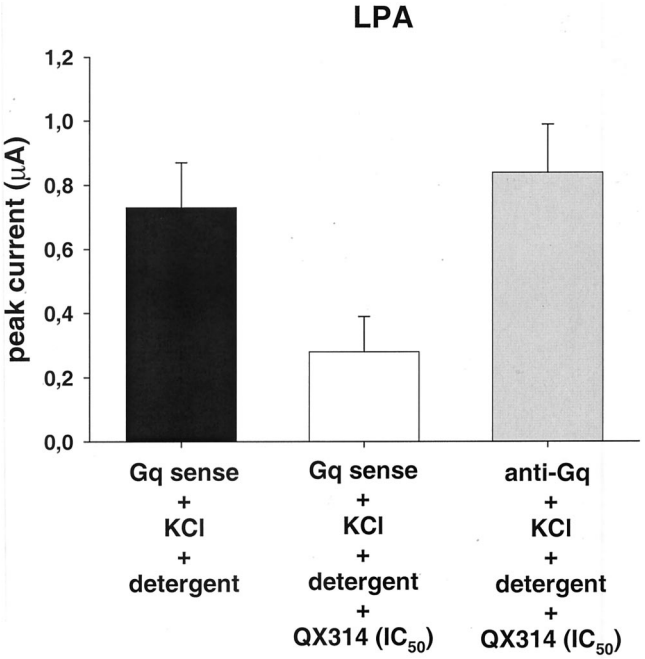

Fig. 3. Mean ± SD of peak currents of LPA responses induced by LPA at one tenth of EC_{50} (29 nM). First bar represents responses of Gq-sense–, KCl-, and detergent carrier–injected oocytes as control group (black bar, 0.73 ± 0.14 μA). Mean response size to 29 nM LPA (one tenth of EC_{50}) after QX314 injection was inhibited to 38.3 ± 15% (white bar). Injection of antisense against the endogenous frog Gq protein restored responses to 115.1 ± 20.5% of the corresponding control response (gray bar).

29 nM LPA (one tenth of EC_{50}), was 0.73 ± 0.14 μA (n = 26). Injection of QX314 (at IC_{50}, 210 μM) reduced LPA-evoked responses to 38% of control response (0.28 ± 0.11 μA; n = 29). This inhibitory effect by QX314 was completely abolished when the oocytes were first injected with anti-Gq (0.84 ± 0.15 μA; n = 33). These results demonstrate that the changed experimental conditions did not affect our previous findings. We now investigated whether intracellular injection of mouse Gq protein would reverse these effects on LPA and muscarinic m3 signaling pathways. Only one tenth of EC_{50} for the agonists was chosen since we knew from previous experiments that, after KCl injection, these concentrations elicit responses similar in size to those of full EC_{50} in the absence of intracellular KCl.

We used Gq-sense oligonucleotide–injected oocytes as control to exclude the possibility that injection of DNA oligonucleotides *per se* affects agonist responses. Mean control response elicited by 29 nM LPA (one tenth of EC_{50}) was 0.6 ± 0.1 μA (n = 42). As shown in figure 4A, injection of antisense oligonucleotides against the endogenous frog Gq protein reduced the mean response size to 33 ± 7% of the corresponding control response (n = 47). In contrast, responses were unchanged (n = 57; 131 ± 29% of control) in cells in addition injected with mouse Gq protein (5×10^{-8} M). Injection of mouse Gq protein "rescued" the inhibitory effect of intracellularly injected QX314 (at IC_{50}, 210 μM). Whereas QX314 was without effect on Gq-depleted oocytes, responses to 29 nM LPA (one tenth of EC_{50}) after QX314 injection were inhibited to 44 ± 10% (n = 54) of the corresponding control response in cells in addition injected with mouse Gq protein (5×10^{-8} M). Thus, mammalian Gq protein both reversed the decreased responses induced by endogenous Gq depletion and enabled the inhibitory effect of intracellular LA.

To assure that this effect is not restricted to the experimental LA QX314, we repeated these studies using intracellularly injected lidocaine (fig. 4B). In Gq-depleted oocytes, mean response size following stimulation with 29 nM LPA (one tenth of EC_{50}) was reduced to 42 ± 11% (n = 44) of control responses (obtained in cells injected with Gq-sense oligonucleotides). Mean control response was 0.8 ± 0.1 μA (n = 39). Additional injection of mouse Gq protein (5×10^{-8} M) prevented this inhibition (response size 109 ± 12% of control; n = 49) and reestablished the intracellular inhibitory effect of lidocaine (at IC_{50}, 150 μM; 53 ± 10% of corresponding control response; n = 48).

To rule out that this effect is specific for the endogenous LPA signaling pathway, we also studied recombinantly expressed m3 muscarinic receptors (fig. 4C). Mean control response was 1.0 ± 0.2 μA (n = 31). Oocytes injected with antisense oligonucleotides directed against endogenous Gq protein showed significantly reduced responses (to 49 ± 10% of control; n = 56). In contrast, responses after additional injection of mouse Gq protein (5×10^{-8} M) were unchanged (94 ± 18% of control; n = 51). These responses were inhibited to 45 ± 16% (n = 56) of control response after intracellular application of QX314 (at IC_{50}, 210 μM).

Discussion

In the current study, we have shown that mammalian Gq protein is able to couple to, and mediate signal transduction through endogenous and recombinantly expressed G-protein–coupled receptors in *Xenopus* oocytes after depletion of the endogenous frog Gq protein. In addition, where knockdown of the endogenous frog Gq protein eliminates sensitivity to intracellular LA of several GPCR pathways, the presence of mammalian Gq protein can prevent this effect.

The Gq subunit is involved in a broad variety of signaling pathways, some of which are of interest to perioperative medicine. Examples of mediators signaling through this G protein are angiotensin, platelet-activating factor, and various cytokines. As a specific example, priming of PMNs, responsible for excessive stimulation of the inflammatory response, is mainly Gq protein mediated.[19] LA inhibition of Gq protein function leads therefore to selective inhibition of PMN priming, explaining at least in part their well-known antiinflamma-

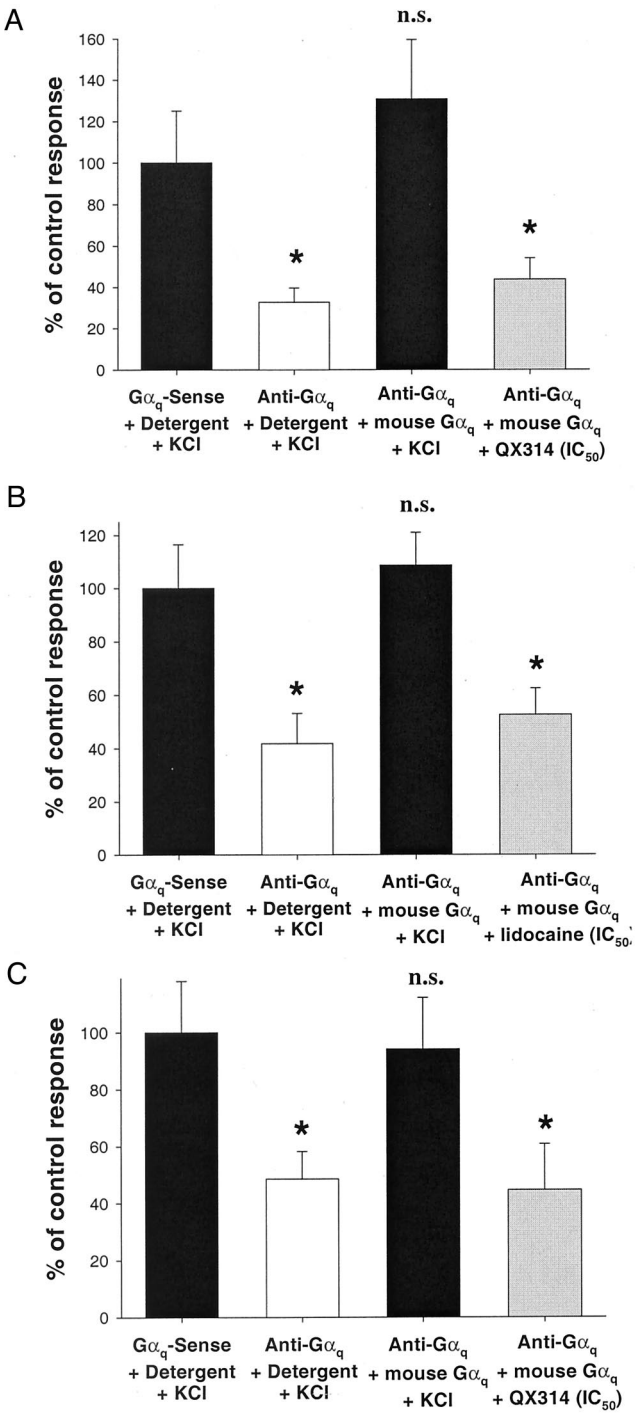

Fig. 4. (*A*) Mean ± SD of peak currents of LPA responses induced by LPA at one tenth of EC_{50} (29 nM). First bar represents responses of Gq-sense–injected oocytes as control group (black bar, 0.6 ± 0.1 μA). Injection of antisense against the endogenous frog Gq protein inhibited responses to 32.6 ± 7.0% of the corresponding control response (white bar). Injection of mouse Gq protein (5×10^{-8} M) restored the reduced response size to 130.8 ± 28.8% as compared with control (third black bar). Fourth bar (gray) illustrates "rescue" of the inhibitory effect of intracellular-injected QX314 (at IC_{50}, 210 μM). Mean response size to 29 nM LPA (one tenth of EC_{50}) after QX314 injection was inhibited to 43.7 ± 10.3%. (*B*) Mean ± SD of peak currents of LPA responses induced by LPA at one tenth of EC_{50} (29 nM). Mean response of Gq-sense–injected oocytes was 0.8 ± 0.1 μA (first black bar). In Gq-depleted oocytes, mean response size was reduced to 41.8 ± 11.3% (white bar). Injection of mouse Gq protein (third black bar) led to a significant increase in mean response size to 108.6 ± 12.4%, and intracellular-injected lidocaine (at IC_{50}, 150 μM) reestablished the intracellular inhibitory effect by the local anesthetic (52.6 ± 9.8%, gray bar). (*C*) Mean ± SD of peak currents of m3 muscarinic responses induced by MCh at one tenth of EC_{50} (20 nM). Mean control response (first black bar) was 1.0 ± 0.2 μA. Anti-Gq injection decreased mean response size to 48.5 ± 9.7% (white bar), whereas mouse Gq protein enhanced m3 signaling to 94.2 ± 18% (third black bar), which was then inhibited by intracellular QX314 (at IC_{50}, 210 μM) to 44.6 ± 16.2% of control response (gray bar).

tory actions (for review, see Hollman and Durieux[3]). In contrast, "physiologic" free radical production by neutrophils is primarily mediated by Gi proteins and therefore not inhibited by LA.

As in our previous studies, we used the *Xenopus* oocyte model. Potential problems with the technique have been discussed in our previous reports.[1,2,6–8] Our results predict that every signaling pathway mediated by Gq would be at least partially inhibited by LAs. However, intravenous administration of LAs does not result in shutdown of major signaling pathways. Several explanations may exist for this apparent paradox. First, the concentrations required for intracellular block of GPCR signaling are significantly greater than those attained in blood after usual intravenous doses. However, additional LA binding sites on GPCRs may result in significantly greater sensitivity,[20] and in some settings (*e.g.*, spinal anesthesia), concentrations able to block Gq signaling are likely to be attained. Second, GPCRs are only partially inhibited by LAs, even at high concentrations. Third, since most receptors couple to multiple G-protein subtypes, lack of activity of the Gq protein can in most instances be compensated for by other G-protein subunits. In particular, G_{11}, structurally very similar and functionally virtually identical to Gq, is likely to play a major role in this regard. Surprisingly, LAs have been shown to discriminate between those two subunits: whereas Gq protein function is inhibited by local anesthetics, G_{11} is not.[1] Indeed, in this and previous studies, local anesthetics interfere selectively with Gq protein function, but not with the function of other Ca-signaling G proteins. LA inhibition of G-protein–gated inwardly rectifying K (GIRK) channels, for example, is not mediated by block of the coupling G_o or Gi protein, but rather by interaction with phosphatidylinositol 4,5-bisphosphate (PIP_2).[21]

Inhibition of mammalian Gq protein function by LAs, as shown in this study, provides a potential explanation for several clinical effects of these compounds[4,22] that cannot be attributed to sodium channel blockade.[3] The antiinflammatory and probably the closely interwoven

antithrombotic effects of LA[3] might be explained at least in part by functional inhibition of a common Gq protein. Platelets in particular might be a target as these cells do not contain G_{11} proteins. Therefore, in these cells, some signaling pathways depend on functioning Gq proteins. Offermanns *et al.*[23] reported that platelets from mice deficient in the α subunit of Gq are unresponsive to a variety of physiologic platelet activators. As a result, these mice are protected from collagen and adrenaline-induced thromboembolism. The authors concluded that the Gq protein may thus be a new target for drugs designed to block the activation of platelets. Together with our data, this suggests that the antithrombotic actions of LAs might result in part from inhibition of Gq in platelets.

To the best of our knowledge, LAs are the first compounds shown to be selective G-protein inhibitors. If the results of this study are confirmed in other models and structural modification of the LA molecule can increase their G-protein–blocking activity while reducing affinity for the sodium channel, new therapeutic indications for these drugs might be feasible.

Summarized, our study has shown that LAs inhibit mammalian Gq protein function. Therefore, next to the Na channel, the Gq protein must be seen as an intracellular target site for LAs, possibly explaining several of their clinical effects. In addition, LAs might be used as lead compounds for the design of novel therapeutics.

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