Time-dependent modulation of muscarinic m1/m3 receptor signalling by local anaesthetics

S. Picardi¹,², M. F. Stevens²,³, K. Hahnenkamp⁴, M. E. Durieux⁵, P. Lirk²,³* and M. W. Hollmann²,³

¹ Department of Anaesthesiology, University Hospital Heidelberg, Im Neuenheimer Feld 110, 69120 Heidelberg, Germany
² Laboratory of Experimental Intensive Care and Anaesthesiology and ³ Department of Anaesthesiology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands
⁴ Department of Anaesthesiology and Critical Care, University Hospital Münster, Albert-Schweitzer-Campus 1, 48149 Münster, Germany
⁵ Department of Anaesthesiology, University of Virginia, Charlottesville, VA, USA

* Corresponding author. E-mail: p.lirk@amc.uva.nl

Editor’s key points
• Local anaesthetics (LAs) may have anti-inflammatory actions.
• G-protein-coupled receptors are likely targets for LAs.
• Xenopus oocytes were used to investigate effects of lidocaine on muscarinic m1 and m3 receptors.
• Lidocaine had a biphasic effect which may be due to modulation by protein kinase C.
• These results may have future clinical implications


Methods. A two-electrode voltage clamp was used to assess the effects of lidocaine or its permanently charged analogue QX314 on recombinantly expressed m1 and m3 receptors in Xenopus oocytes. Antisense knock-down of functional Gq protein and inhibition of protein kinase C (PKC) served to define mechanisms and sites of action.

Results. Lidocaine affected muscarinic signalling in a biphasic way: an initial decrease in methylcholine bromide-elicited m1 and m3 responses after 30 min, followed by a significant increase in muscarinic responses after 8 h. Intracellularly injected QX314 time-dependently inhibited muscarinic signalling, but had no effect in Gq-depleted oocytes. PKC-antagonism enhanced m1 and m3 signalling, but completely abolished the LA-induced increase in muscarinic responses, unmasking an underlying time-dependent inhibition of m1 and m3 responses after 8 h.

Conclusions. Lidocaine modulates muscarinic m1 and m3 receptors in a time- and Gq-dependent manner, but this is masked by enhanced PKC activity. The biphasic time course may be due to interactions of LAs with an extracellular receptor domain, modulated by PKC activity. Prolonged exposure to LAs may not benefit pulmonary function, but may positively affect postoperative cognitive function.

Keywords: G-protein-coupled receptors; local anaesthetics; muscarinic m1 and m3 receptors

Accepted for publication: 11 June 2013

Block of voltage-gated sodium channels, leading to inhibition of nerve impulse conduction, is probably the most important effect of local anaesthetics (LAs) and the major mechanism underlying their well-known antinociceptive and antiarrhythmic properties. However, recently discovered aspects of LA function, in particular their anti-inflammatory properties, seem to provide further significant benefit for patients in the clinical setting, as they attenuate the surgery-induced stress response but do not impair physiological host defence.¹ Signal ling of G-protein-coupled receptors (GPCRs) and in particular the α-subunit of G-proteins of the Gq/11 family, well known for their role within the immune response, seem to be one potential target for LAs.² ³

We have previously shown that signalling of different immunomodulatory Gq-coupling receptors, such as the lysophosphatidic acid (LPA) or thromboxane A₂ receptor, is time-dependently inhibited at concentrations of LA similar to that seen clinically after epidural anaesthesia or i.v. administration.⁴ An increased inhibitory potency and thus a potentially increased efficacy on GPCR signalling pathways after prolonged exposure to LA might thus explain the beneficial effects, such as faster return of gastrointestinal function or...
improved postoperative pain, seen in the clinical setting even after completion of LA treatment.\(^5\)

Functional \(\text{G}_{\alpha q}\)-protein was critically required for the observed time-dependent effects of long-term LA exposure, suggesting that all GPCRs of the Gq/11-family would be time-dependently inhibited.\(^3\) However, in pilot studies, m1 and m3 muscarinic acetylcholine receptors, both bound to the Gq/11 family of G proteins, behaved differently. m1 and m3 muscarinic receptors were shown to be more sensitive to LA than the neuronal sodium channel: half-maximal inhibitory concentrations \((IC_{50})\) were 18 nM for the m1 receptor and 370 nM for the m3 receptor, respectively, which is \(\sim\)100–1000 times less than required for sodium channel block \((IC_{50} = 60–200 \mu M).\(^6\) The \(\text{G}_{\alpha q}\)-protein was defined as the site of action on these receptors for short-term exposure effects of LA.\(^2\) Signalling of muscarinic receptors plays an essential role in memory and learning, and is a potential target for the treatment of cognitive deficits.\(^8\) Persistent cognitive impairment after surgery is an increasing challenge, particularly in elderly patients, who have higher morbidity and mortality. I.V. lidocaine was shown to improve early post-operative dysfunction in patients undergoing cardiac surgery.\(^10\)

Muscarinic m1 and m3 receptors are widely expressed in airway and lung tissue, mediating alveolar smooth muscle contraction and thus airway hyperresponsiveness.\(^11\) Previous studies demonstrated that nebulized lidocaine improved pulmonary function or mitigated bronchoconstriction after anaesthetic induction and intubation in asthmatic patients when given i.v.\(^12\)\(^13\) Since m1 and m3 muscarinic receptors play an important role clinically, modulation of their signalling may be useful. We aimed to characterize the effects of long-term exposure to LA on muscarinic signalling, and the underlying mechanisms of action, in \(Xenopus\) oocytes.

**Methods**

All animal experiments were approved by the Animal Research Committee, University of Maastricht, The Netherlands.

**Oocyte experiments**

Oocyte harvesting and receptor expression were performed as described previously.\(^6\) Briefly, oocytes were obtained from anaesthetized female \(Xenopus\) laevis frogs, defolliculated and injected with rat m1 and m3 muscarinic acetylcholine receptor complementary RNA. Agonist-induced calcium-activated chloride currents \(I_{\text{Cl}(Ca)}\) were measured using two-electrode voltage clamping (sample traces are shown in Fig. 1a and b). \(Xenopus\) laevis frogs were housed in an established frog colony and fed regular frog brittle twice weekly. Surgery for oocyte harvesting was performed once every 2 months.

**Drug administration**

Agonists and LA were used at concentrations used in earlier studies.\(^6\)\(^7\) Acetyl-\(\beta\)-methylcholine bromide (MCh) was used as an agonist for the m1 and m3 muscarinic receptors, diluted in Tyrode’s solution (150 mM NaCl, 5 mM KCl, 1 mM MgCl\(_2\), 6 mM Na\(_2\)HPO\(_4\), 2 mM CaCl\(_2\), 2 mM HEPES, 10 mM dextrose, 10 mM HEPS) to the desired concentrations and superfused over the oocyte for 10 s. The oocyte was placed next to the inflow tubing to ensure complete exposure to agonists, treatment, or both. Las were diluted in Barth’s solution to concentrations corresponding to 1/10th of the previously determined IC\(_{50}\).\(^6\)\(^7\) For extracellular administration of LA, oocytes were incubated for different durations in plain Barth’s solution with or without LA. Intracellular administration of QX314 was performed as described previously.\(^7\) Control and treatment responses were obtained in different oocytes to avoid an alteration of measurements by receptor desensitization.

**Oligonucleotide injection**

Phosphorothioate oligonucleotides were synthesized by the University of Virginia Research Facility. The antisense sequence is complementary to specific 20-base segments with \(<50%\) homology with other types of \(X.\) laevis G-proteins.\(^14\) Sense oligonucleotides were used as control. Oocytes were injected with 50 nl sterile water containing 50 ng per cell antisense or sense oligonucleotides; 24 h after oligonucleotide injection, the cells were tested as described previously.\(^7\)

**Experiments using PKC or phosphatase inhibitors**

The PKC antagonists chelerythrine (CT, 10 \(\mu M\), targeted to the substrate binding site of PKC) or bisindolylmaleimide (BIM, 10 \(\mu M\) a competitive PKC antagonist for adenosine triphosphate binding to the catalytic domain) were used. As described previously, the rather high concentrations used were chosen in order to ensure the inhibition of all PKC isoforms present in the \(Xenopus\) oocyte.\(^6\) For the inhibition of receptor-dephosphorylation, we used the phosphoserine/phosphothreonine phosphatase inhibitor okadaic acid (1 \(\mu M\)). Oocytes were incubated in PKC or phosphatase inhibitors for 1 h before administration of LA and/or agonists to assure appropriate inhibition. Responses elicited by stimulation of m1 or m3 receptors by MCh (at EC\(_{50}\) in the presence of LA and the PKC or phosphatase antagonists were normalized to control responses obtained from oocytes that were incubated only with the corresponding PKC or phosphatase antagonist.

**Binding experiments**

Membrane preparation and ligand binding studies were performed as described previously.\(^7\) In brief, Chinese hamster ovary (CHO) cells, stably transfected with the m3 muscarinic receptor, were homogenized. Receptor density and equilibrium dissociation constants in CHO cell membranes were determined by specific binding of \([\text{\(^{3}\}H}\) radiolabelled quinuclidinyl benzylate (\([\text{\(^{3}\}H}\)QNB) (0.1–16 nM), a muscarinic receptor agonist, and measured by a scintillation counter. Non-specific binding was identified by the addition of 5 \(\mu M\) atropine to displace specific binding of \([\text{\(^{3}\}H}\)QNB. Five different membrane preparations, assayed with the same batch of radioligand, were used for each time point.

**Materials**

Molecular biology reagents were obtained from Promega (Madison, WI, USA), CHO cells (CRL-1982), stably transfected...
with the rat muscarinic m3 receptor, were purchased from ATCC (Manassas, VA, USA) and other chemicals were obtained from Sigma (St Louis, MO, USA). QX314 was a gift from Astra Pharmaceuticals, L.P. (Westborough, MA, USA).

Statistical analysis

Results are reported as mean and standard deviation (SD). At least 22 oocytes were used to determine each data point. As variability between batches of oocytes is common, responses were normalized to control response. Statistical comparisons were made using one-way analysis of variance followed by the Dunnet correction for multiple comparisons. \( P < 0.05 \) was considered significant. SigmaStat 2.0 (Jandel Scientific Corporation, San Rafael, CA, USA) was used for all statistical analyses.

Results

Functional expression of m1 and m3 muscarinic receptors in Xenopus oocytes

Whereas untreated oocytes did not respond to MCh, a transient increase in \( I_{\text{Cl(Ca)}} \) was seen after MCh stimulation in oocytes expressing the m1 and m3 muscarinic receptor. Evidence that signalling of these specific receptors was responsible for responses was shown in earlier studies since atropine and pirenzepine attenuated muscarinic m1 signalling and 4-diphenylacetoxy-N-methylpiperidine, an m3 preferring cholinergic receptor antagonist, inhibited m3-mediated signal transduction.\(^{15,16}\)

Lidocaine modulates m1 and m3 muscarinic signalling in Xenopus oocytes biphasically

Incubation of oocytes which recombinantly expressed m1 muscarinic receptors for various durations with lidocaine (1.8 nM) induced a biphasic effect on m1 signal transduction. Whereas lidocaine significantly inhibited MCh (0.57 \( \mu \text{M} \))-induced responses of the m1 receptor after 30, 60, and 120 min longer exposure to the LA (\( \geq 8 \) h) resulted in a small but significant increase in MCh-elicited responses than compared with control (Fig. 1c). A biphasic effect was also observed when oocytes expressing the m3 receptor where incubated with lidocaine (37 nM, \( n = 25 \)). Effects were determined at different time points and are shown as mean (SD), responses, normalized to corresponding control responses. *Significance \( (P < 0.05) \) compared with the control group.

Fig 1 Modulation of m1 and m3 signalling by extracellular lidocaine in Xenopus oocytes. (a) Sample traces of \( I_{\text{Cl(Ca)}} \) induced by 10 s administration of MCh at EC\(_{50}\) in an oocyte expressing the m3 receptor and in the presence of lidocaine at IC\(_{50}\). Peak currents are 1.12 mA (a) and 0.48 mA (b). (c) Effects of long-term exposure to lidocaine (1.8 nM) on m1 signalling (\( n = 22 \)). Recombinant expressed m1 receptors were stimulated with MCh (0.57 \( \mu \text{M} \)). (c) Lidocaine (37 nM) on m3 responses, elicited by MCh (0.42 \( \mu \text{M} \), \( n = 25 \)). Effects were determined at different time points and are shown as mean (SD), responses, normalized to corresponding control responses. *Significance \( (P < 0.05) \) compared with the control group.
Functional ligand binding of recombinantly expressed m3 receptors in CHO cells

To exclude the possibility of muscarinic receptor dysfunction as an effect of time ([3H]QNB), binding to membranes prepared from CHO cells, stably transfected with the rat m3 muscarinic receptor, was studied at different time points. Free drug-specific binding was saturable and reached its maximum at 2.2–4.6 nM (Fig. 2). The saturation curves and Scatchard analyses confirm near steady dissociation constants ($K_d$) and receptor densities ($B_{max}$) over time, suggesting muscarinic m3 receptors to be fully functional even after longer study times in our model (Table 1).

Intracelularly injected QX314 inhibits m1 and m3 signalling in a time- and G-protein dependent manner

Since time-dependent inhibition of different Goq-coupling receptors by LA has been shown to have an intracellular site of action, we studied the effects of the permanently charged

---

**Fig 2** Functional ligand binding of recombinantly expressed m3 receptors in CHO cells. [3H]quinuclydinyl benzylate ([3H]QNB) (0.1–16 nM) binding to membranes prepared from Chinese ovarian hamster cells stably transfected with the rat muscarinic m3 receptor at different time points (n= 5 at each time point).
lidocaine analogue QX314, administered intracellularly. In contrast to lidocaine, QX314 (96 μM for m1 and 45 μM for m3) inhibited MCh (0.57 and 0.42 μM, respectively)-induced m1 and m3 signal transduction in a time-dependent manner (Fig. 3A and B, respectively) after 48 h.

To ascertain if modulation of Gαq-protein function might be a potential site of action for this QX314-induced time-dependent effect on muscarinic signalling, we next studied the inhibitory potency of intracellularly injected QX314 (45 μM) on MCh-elicited m3 responses in oocytes after depletion of Gαq-protein using antisense oligonucleotides. m3 receptors were used for antisense studies as they gave more robust receptor expression after multiple microinjections. According to earlier data on LA effects on LPA signal transduction, depletion of Gαq-protein fully abolished the observed QX314 effects on muscarinic signalling at all time points studied (Fig. 3C), indicating that time-dependent inhibition by intracellular QX314 is critically dependent on Gαq-protein function.

Effects of extracellularly applied lidocaine and QX314 on m3 receptors in Gαq-depleted oocytes

The effects of lidocaine and QX314 on m1 and m3 signalling were clearly different. As lidocaine reaches both the intracellular and extracellular space, this may be due to an extracellular

---

**Table 1** Radioligand binding of recombinantly expressed m3 receptors in Chinese ovarian hamster cells at different time points. Data are shown as mean and SD. *Kd*, dissociation constant; *Bmax*, receptor density.

<table>
<thead>
<tr>
<th>Time points</th>
<th>Control</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kd (nM)</td>
<td>0.148 (0.03)</td>
<td>0.152 (0.04)</td>
<td>0.152 (0.03)</td>
<td>0.147 (0.03)</td>
<td>0.154 (0.04)</td>
</tr>
<tr>
<td>Bmax (fmol mg⁻¹ protein)</td>
<td>3545 (217)</td>
<td>3479 (225)</td>
<td>3657 (199)</td>
<td>4650 (233)</td>
<td>4760 (231)</td>
</tr>
</tbody>
</table>

---

**Fig 3** Sites of action for LA effects on muscarinic signalling. Effects of intracellularly injected QX314 (96 μM for m1 and 45 μM for m3) on (a) m1 and (a) m3 signalling (n=25 each). Responses were elicited by stimulation with MCh (0.57 μM for m1 and 0.42 μM for m3 receptors). (c) Effects of intracellularly administered QX314 (45 μM) on m3 signalling after depletion of the Gαq-protein (n=22). (c) Effects of extracellularly applied lidocaine (37 nM) on m3 responses, induced by MCh in Gαq-depleted oocytes (n=24). Data are mean (SD), normalized to corresponding control responses. *Significance (P<0.05) compared with the control group.
target site, accessible to lidocaine but not to the permanently charged QX314. To confirm this, we studied the effects of extracellularly administered lidocaine on m3 responses in Goq-protein-depleted oocytes. Extracellular lidocaine (37 nM) inhibited m3 signalling by ~20% after the first hour (Fig. 3d). This attenuation of signalling results from interaction with an additional extracellular binding site for uncharged LA at the m3 receptor. However, further incubation of Goq-protein-depleted oocytes with lidocaine (>4 h) enhanced m3 signalling even more than in the presence of functional Goq-protein.

Thus, the observed biphasic effect of LA on muscarinic signalling seems to consist of two opposing parts: an intracellular time- and Goq-protein-dependent inhibition and an even slower extracellular time-dependent signalling enhancement.

**PKC antagonism abolishes the lidocaine-induced time-dependent increase in m1 and m3 signalling**

The slow time course of signalling enhancement suggests covalent modification of the receptor rather than allosteric modulation. Receptor phosphorylation might be one possible mechanism and so we investigated the role of PKC in lidocaine-induced increased muscarinic responses, using two different PKC antagonists.

In the absence of LA, inhibition of PKC increased m1 and m3 receptor signalling by ~25% of control response after 49 h (1 h pre-treatment and 48 h study period, data not shown). When both lidocaine (1.8 nM for m1 and 37 nM for m3) and BIM (10 μM) were present, MCh-induced muscarinic signalling was inhibited in a time-dependent manner (Fig. 4a and b). Similar results were obtained using lidocaine and CT (Fig. 4c and d).

**Phosphatase antagonism does not affect the increase in m1 and m3 responses after long-term exposure to lidocaine**

As receptor phosphorylation seems to play an important role in the biphasic effect of lidocaine on muscarinic signalling, we next questioned if inhibition of dephosphorylation using the phosphoserine/phosphothreonine phosphatase inhibitor okadaic acid resulted in even further enhancement of signalling.

Pre-treatment of oocytes in okadaic acid (1 μM) for 1 h did not have any effect on MCh-elicited muscarinic responses (data not shown).

---

**Fig 4** PKC antagonism modulates the effect of lidocaine on muscarinic signalling. Effects of extracellularly applied lidocaine on (a) m1 and (a) m3 signalling (1.8 and 37 nM, respectively), stimulated by MCh (0.57 μM for m1 and 0.42 μM for m3 receptors) after pre-treatment with BIM (10 μM, n = 25) or (c and d) CT (10 μM, n = 25), for 1 h. Data are mean (SD) normalized to corresponding control responses. *Significance (P < 0.05) compared with the control group.
shown). However, when cells were additionally treated with lidocaine (1.8 nM), m1 signalling was initially inhibited in a time-dependent manner, followed by a maximal increase in signalling up to 150% of control response after 48 h. A similar biphasic effect in the presence of okadaic acid and lidocaine (37 nM) was seen for m3 signal transduction (data not shown).

**PKC antagonism abolishes the time-dependent increase in m1 and m3 signalling by extracellular QX314**

To further characterize the site of action for LA to enhance muscarinic signalling after a certain time, we next studied the effects of the PKC antagonists BIM or CT and/or extracellularly applied QX314 (500 µM) on MCh-elicited m3 responses. As shown in Figure 5A and B, 1 h pre-treatment with each PKC antagonist alone increased m3 signalling by ~25% for BIM and 35% for CT. With respect to QX314, m3 responses were not affected for the first 2 h, but increased after 4 h (effects of QX314 were determined for oocytes of each series of PKC antagonists). This increase was completely abolished when one of the PKC antagonists, either BIM or CT, was already present, suggesting that LA interact with an extracellular site at the m3 receptor and, as similar results were obtained for m1 signalling (data not shown), with an extracellular site at the m1 receptor, accordingly.

**Intracellular lidocaine does not enhance m3 signalling**

Direct interaction with PKC could be another potential mechanism of LA to time-dependently enhance muscarinic signalling. To confirm this hypothesis, we injected lidocaine (500 µM) into oocytes expressing the m3 receptor and prevented diffusion to the extracellular space by vigorously}

---

**Fig 5** PKC-dependent modulation of muscarinic m3 signalling by extra- and intracellular LAs. Time course of m3 responses after incubation with BIM (10 µM) or CT (10 µM), QX314 (500 µM) or the combination of QX314 (500 µM) and BIM or CT. (a) Data obtained in the presence of BIM (n = 25) and (b) data when CT was used (n = 25). Oocytes were pre-treated with PKC antagonists for 1 h, followed by incubation with QX314 for 48 h. Responses were elicited by MCh (at EC50). (c) Effects of intracellularly applied lidocaine on m3 signalling in Gaq-protein-depleted oocytes (n = 24). Receptors were stimulated with MCh (at EC50). Data are mean (so) normalized to corresponding control responses.
superfusing the oocyte with Tyrode’s solution. To furthermore prevent lidocaine-induced modulation of \( \text{Ga}q \)-protein function, we used oocytes which had been depleted of intact \( \text{Ga}q \)-protein by antisense oligonucleotides. As expected, intracellular lidocaine did not have any effects on m3 signalling, as shown in Figure 5c.

Discussion

This study shows that m1 and m3 muscarinic signalling is time-dependently modulated by LA. However, muscarinic responses are affected by two opposing mechanisms: an initial inhibition is mediated by an intracellular site of action, requiring functional \( \text{Ga}q \)-protein, followed by enhanced signalling based on interference with an extracellular receptor domain modulated by PKC and protein phosphatase activity, which masks the inhibitory potencies of LA.

Many clinical trials have demonstrated beneficial anti-inflammatory effects of LA perioperatively and we previously showed that LA inhibiting signalling of several \( \text{Gq/11} \) class GPCRs, a \( \text{G} \) protein family that predominantly mediates haemostatic and inflammatory signalling, in a time- and \( \text{Gq/11} \)-dependent manner.9

Besides modulation of immune responses, LAs were also shown to positively affect bronchial hyperreactivity and post operative cognitive function.10 12 13 Muscarinic m1 receptors, found in the central nervous system, are involved in processing memory and learning. Muscarinic m3 receptors, expressed in smooth muscle cells of the respiratory tract, mediate bronchoconstriction and thus bronchiolar tone. Both may be a potential target site for LA.9 11 Since both receptors couple to \( \text{Ga}q \)-protein, we anticipated their signalling to be inhibited by LA in a similar manner to the other GPCRs, and thus the observed increase in signalling was rather unexpected.

Muscarinic receptors are known to be regulated by PKC activity. Whereas phosphorylation results in desensitization of receptor function, inhibition of PKC enhances muscarinic responses.17 In line with Shiga and colleagues,18 we observed an increase in MCh-elicited signals by \( \sim 25\% \) in oocytes treated with PKC-antagonists, BIM, or CT, when compared with control, suggesting PKC involvement.

However, pre-treating oocytes with PKC antagonists before incubation with lidocaine prevented the late increase in muscarinic responses, and unmasked the underlying time-dependent inhibition of muscarinic signalling by LA. These findings suggest that receptor phosphorylation, dephosphorylation, or both play a key role in the biphasic effect of lidocaine on muscarinic signalling. Confirmatory data were obtained when okadaic acid by antisense oligonucleotides. As expected, intracellular lidocaine did not have any effects on m3 signalling, as shown in Figure 5c.

Thus, up-regulation of receptor function after prolonged exposure to LA seems to be because of PKC activity and receptor phosphorylation. As PKC is located intracellularly, we assumed a direct interaction of LA with the enzyme. However, intracellular injection of QX314 did not have any stimulating effects on muscarinic signalling, suggesting an extracellular site of action for the biphasic LA effect, and no direct interactions with PKC. Since QX314 is a permanently charged lidocaine analogue, the lack of effect did not exclude an extracellular binding site for uncharged LA, although as intracellular administration of lidocaine in oocytes depleted of functional \( \text{Ga}q \)-protein did not have any effect on muscarinic signalling, this possibility was dismissed.

As in our earlier studies, we used the Xenopus oocyte model for our studies. Although the cells have a long survival time in vitro and are useful to study intracellular actions,2 6 7 20 there are several potential problems with the technique. Actual PKC activity is often hard to determine in Xenopus oocytes and so altered calcium-activated chloride currents were used as a surrogate in the presence of PKC inhibitors. Although recombinant expression of a single subtype of muscarinic receptors allows for mechanistic studies due to easy separation of signalling pathways, it is rather difficult to make a statement on how lidocaine-induced modulation of G proteins other than \( \text{Ga}q/11 \) would affect the effects on muscarinic m1 and m3 receptors. We have previously shown that LA enhances signalling of the \( \text{Gq}i \) coupling human adenosine 1 receptor, leading to a decrease in cAMP levels.21 However, even in the presence of muscarinic m2 and m4 receptors, lidocaine will most likely modulate m1 and m3 signalling as described above, since both receptors primarily couple to \( \text{Gq/11} \).

Other LAs are likely to affect muscarinic signalling in the same way. However, as the efficacy of LA to modulate GPCR signalling depends on their physicochemical properties, further studies are required to determine the size of effect of other LAs on muscarinic m1 and m3 signal transduction.22

Taken together, our data suggest that prolonged exposure of muscarinic m1 and m3 receptors to LA may result in a change of receptor confirmation: new phosphorylation sites, usually not accessible for PKC, may become available and as a consequence, these receptors are phosphorylated by basal PKC activity. Under these circumstances, PKC may induce up-regulation of receptor function instead of the expected down-regulation (Fig. 6). Direct interaction between LA and PKC may not be obligatory to result in the biphasic effect on muscarinic signalling observed after prolonged exposure.

In conclusion, LAs affect muscarinic signalling via PKC-dependent mechanisms, yet without direct interference with the enzyme. One important finding of the present study is that LAs do not have to directly modulate PKC in order to induce PKC-mediated effects. In addition, PKC activity does not always induce predictable effects under these circumstances. Based on these data, not all GPCRs that signal through \( \text{Gq/11} \) may be time-dependently inhibited by LA. Prolonged exposure to LA may not result in prolonged inhibition of muscarinic signalling. For clinical practice, this may mean that prolonged exposure to LA may not benefit pulmonary function, but it may benefit cognitive deficits. However, this remains to be determined in clinical trials.
**Authors’ contributions**


**Acknowledgement**

We gratefully acknowledge Prof. Dr Eike Martin, MD, PhD (Professor of Anaesthesiology and Chair, Department of Anaesthesiology, University of Heidelberg, Heidelberg, Germany) for his support.

**Declaration of interest**

None declared.

**Funding**

This work was supported by a M.D.-medical research trainee grant from the Netherlands Organisation for Health Research and Development (ZonMW AGIKO to S.P.), by the Medical
References

7. Hollmann MW, Ritter CH, Henle P, de Klayer M, Kamatchi GL, Durieux ME. Inhibition of m3 muscarinic acetylcholine receptors by local anesthetics. Br J Pharmacol 2001; 133: 207–16
15. Nietgen GW, Hoenemann CW, Chan CK, Kamatchi GL, Durieux ME. Volatile anesthetics have differential effects on recombinant m1 and m3 muscarinic acetylcholine receptor function. Br J Anaesth 1998; 81: 569–77
16. Durieux ME. Halothane inhibits signaling through m1 muscarinic receptors expressed in Xenopus oocytes. Anesthesiology 1995; 82: 174–82

Handling editor: H. F. Galley