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

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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 invesitgate effects of lidocaine on muscarinic m1 and m3 receptors.
- Lidocine had a biphasic effect which may be due to modulation by protein kinase C.
- These results may have future clinical implications

Background. Signalling of several G-protein-coupled receptors of the Gq/11 family is time-dependently inhibited by local anaesthetics (LAs). Since LA-induced modulation of muscarinic m1 and m3 receptor function may explain their beneficial effects in clinical practice, such as decreased postoperative cognitive dysfunction or less bronchoconstriction, we studied how prolonged exposure affects muscarinic signalling (Wang D, Wu X, Li J, Xiao F, Liu X, Meng M. The effect of lidocaine on early postoperative cognitive dysfunction after coronary artery bypass surgery. *Anesth Analg* 2002; **95**: 1134–41; Groeben H, Silvanus MT, Beste M, Peters J. Combined lidocaine and salbutamol inhalation for airway anesthesia markedly protects against reflex bronchoconstriction. *Chest* 2000; **118**: 509–15).

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 Gαq-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 Gαq-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 Gαq-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

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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] Signalling 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 Gαq-coupling receptors, such as the lysophosphatidic acid (LPA) or thromboxane A_2 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 Gαq-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.[4] 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 ~100–1000 times less than required for sodium channel block (IC_{50} is 60–200 μM).[6–8] The Gαq-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.[9] 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 postoperative 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 [$I_{Cl(Ca)}$] 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-β-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 $MgCl_2 \cdot 6H_2O$, 2 mM $CaCl_2 \cdot 2H_2O$, 10 mM dextrose, 10 mM HEPES) 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.[4] 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 μM, targeted to the substrate binding site of PKC) or bisindolylmaleimide (BIM, 10 μM 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.[4] For the inhibition of receptor-dephosphorylation, we used the phosphoserine/phosphothreonine phosphatase inhibitor okadaic acid (1 μM). 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 [^{3}H] radiolabelled quinuclydinyl benzylate ([^{3}H]QNB) (0.1–16 nM), a muscarinic receptor antagonist, and measured by a scintillation counter. Non-specific binding was identified by the addition of 5 μM atropine to displace specific binding of [^{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