Regulation of Cyclic AMP Accumulation by Lysophosphatidic Acid in Human Airways Smooth Muscle Cells from Normal and Asthmatic Donors

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Lysophosphatidic acid (LPA) is a bioactive lipid mediator with demonstrated roles in airways function and disease (Tager et al., 2008). LPA exerts its actions through activation of a family of cognate G protein-coupled receptors, which couple to a variety of G proteins, including Gαi, to inhibit adenylyl cyclase (AC) activity. Cyclic AMP is a known regulator of airways smooth muscle tone and this second messenger system is a primary target of treatments to alleviate the airway smooth muscle hypercontractility associated with asthma. In this study we have asked whether differences exist in the regulation of cyclic AMP accumulation by LPA in low passage (P2-P4) human airways smooth muscle (hASM) cells derived from 6 normal (N) and 10 asthmatic (A) donors.

No significant differences were found in basal cyclic AMP levels in N- and A-derived hASM (N, 17 ± 3; A, 38 ± 8 pmol mg⁻¹ protein). Addition of the AC activator, forskolin (10 µM), for 10 min caused marked increases in cyclic AMP accumulation (N, 644 ± 75; A, 932 ± 185 pmol mg⁻¹ protein). Pre-addition of LPA (for 5 min) caused concentration-dependent decreases in cyclic AMP accumulation in response to subsequent forskolin addition, with a maximally-effective concentration of LPA (3 µM) causing 56 ± 7% and 50 ± 6% reductions in forskolin-stimulated cyclic AMP accumulation in N- and A-derived hASM cells, respectively. The inhibition caused by LPA could be completely blocked by incubation with the LPA₁/₃ receptor-selective antagonist, Ki16425 (Ohta et al., 2003). Addition of the β-adrenoceptor agonist, isoprenaline (1 µM), resulted in a significantly greater cyclic AMP accumulation in A- versus N-derived hASM cells (N, 504 ± 56; A, 761 ± 115 pmol mg⁻¹ protein; P<0.05). Pre-incubation with a range of LPA concentrations (0.1-3000 nM) followed by addition of isoprenaline (1 µM) results in a biphasic response with lower LPA concentrations reducing isoprenaline-stimulated responses (by a maximum of (N) 27 ± 9% and (A) 21 ± 5%), but higher LPA concentrations increasing the response (to (N) 144 ±19% and (A) 137 ± 9%) compared to the effects of isoprenaline alone. Pre-incubation with Ki16425 blocked both the inhibitory and stimulatory phases of the LPA modulation.

The biphasic LPA modulation of the isoprenaline response observed here could be recapitulated in a model cell-line (SW982), which endogenously expresses only the LPA₁ receptor subtype. Furthermore, mechanistic studies in the SW982 cell-line have shown that pertussis toxin eliminates the inhibitory phase, but not the stimulatory phase of the LPA modulatory effect, and the modulation was largely insensitive to removal of extracellular Ca²⁺. While our study has highlighted some significant differences in cyclic AMP turnover in hASM cells derived from normal and asthmatic donors, no major differences in the modulatory actions of LPA have been observed.