

Formaldehyde induces Rho-associated kinase activity to evoke airway hyperresponsiveness.

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ONLINE DATA SUPPLEMENT

Supplementary Materials and Methods

Determination of toxicity in PCLS

PCLS were exposed to PBS or formaldehyde as described in Materials and Methods. For inducing maximal tissue damage in slices, some slices were incubated overnight in 10% Triton X 100. One hundred microliter of the supernatants, in triplicates, was screened for LDH activity using the LDH detection kit (Cayman Chemical, Ann Arbor, MI) following manufacturer's instructions. LDH activities in each treatment were expressed as percentage of the maximal LDH release in Triton-x-100-treated slices.

Rho Inhibition in HASM cells

To determine whether formaldehyde-induced MYPT1 phosphorylation is mediated through the upstream small protein Rho, HASM cells were incubated with 1 ug/ml Rho Inhibitor I (Exoenzyme C3 transferase from *C. botulinum*, Cytoskeleton Inc, Denver, CO) for 4 h. Then, cells were exposed to PBS or formaldehyde as described in Materials and Methods. Cell lysates were collected 24 h post-exposure to formaldehyde, in 0.6 M HClO₄ for detection of pMYPT1. As positive controls (to induce MYPT1 phosphorylation), some of the Rho Inhibitor I-treated cells were briefly exposed to 10 μM carbachol (Cch) for 10 min and cell lysates were collected.

Supplementary Figure Legends

Figure S1: Formaldehyde has little effect on thrombin-induced $[Ca^{2+}]_i$ in HASM cells

HASM cells exposed to PBS or formaldehyde were loaded with Ca^{2+} -binding fluo-8 dye and stimulated with 1 U of human recombinant thrombin. A) Basal and thrombin-induced Ca^{2+} transients were comparable between PBS- and formaldehyde-treated HASM cells (60 cells/treatment from n=3 donors). B) Average area under the curves (AUC) derived from thrombin-induced Ca^{2+} transients were comparable between PBS and formaldehyde-treated HASM cells.

Figure S2: Formaldehyde-induced MYPT1 phosphorylation is Rho-independent

HASM cells were exposed to PBS or formaldehyde in the presence of vehicle (Veh) or 1 ug/ml Rho Inhibitor I. Lysates were probed for MYPT1 phosphorylation 24 h post-exposure. Positive controls were exposed to 10 μ M carbachol (Cch) for 10 min in the presence of Veh or Rho Inhibitor I. A) Representative blot (n=4 donors) shows that Cch-induced MYPT1 phosphorylation is significantly inhibited by Rho Inhibitor, with little effect on formaldehyde-induced MYPT1 phosphorylation. B) Densitometry ratio between pMYPT1 and tubulin (Mean \pm SEM, n=4 donors) shows that Rho Inhibitor has little effect on formaldehyde-induced MYPT1 phosphorylation in HASM cells. Cch-induced MYPT1 phosphorylation is significantly inhibited by Rho Inhibitor (*p=0.043, n=4 donors).

Supplementary Figures

Figure S1: Formaldehyde has little effect on thrombin-induced $[Ca^{2+}]_i$ in HASM cells

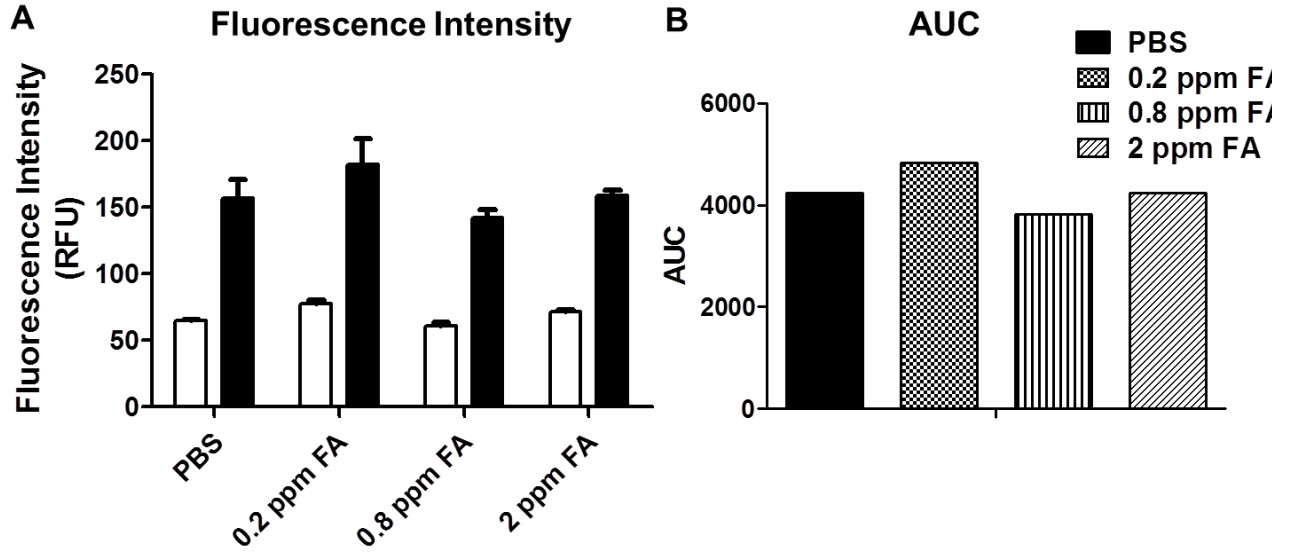


Figure S2: Formaldehyde-induced MYPT1 phosphorylation is Rho-independent

