# Epinephrine Induces Human Airway Smooth Muscle Contraction Through the Alpha-1 Adrenergic Receptor After Beta-2 Adrenergic Receptor Desensitization

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## Abstract

**Rationale**: Human airway smooth muscle (HASM) cells express $\beta_2$ adrenergic receptors ($\beta_2$AR) that promote airway smooth muscle relaxation, and $\alpha_1$ adrenergic receptors ($\alpha_1$AR), that may evoke smooth muscle contraction. Little research has been done to determine if the $\alpha_1$AR plays a role in evoking bronchoconstriction. Epinephrine (EPN), an adrenergic receptor agonist, binds to α and β adrenergic receptors. We posit that $\alpha_1$AR agonists induce bronchoconstriction after desensitization of the $\beta_2$AR in HASM cells.

**Methods**: RNA sequencing was performed on asthmatic and non-asthmatic HASM cells. Human tracheal rings were immunostained for $\alpha_1$AR. HASM cells were treated overnight with $\beta_2$ agonist to desensitize the $\beta_2$AR and were then stimulated with EPN either with or without pretreatment with Doxazosin mesylate, an $\alpha_1$AR antagonist. Lysates were then collected for immunoblot. Blots were probed for phosphorylated myosin light chain (pMLC) as the surrogate for bronchoconstriction.

**Results**: RNAseq and immunostaining of tissue from human trachea show high levels of $\alpha_1$AR mRNA and protein, respectively. EPN increases MLC phosphorylation (2.16 ± 1.27 fold change non-$\beta_2$AR desensitized vs $\beta_2$AR desensitized, p=0.0009) and cytosolic calcium levels (AUC 365,799 ± 25,777 Control vs 474,587 ± 74,065 $\beta_2$AR desensitized) of HASM cells after $\beta_2$AR desensitization. Doxazosin mesylate abrogates both MLC phosphorylation (0.57 ± 0.08 fold $\beta_2$AR desensitized+Doxazosin+EPN vs $\beta_2$AR desensitized+EPN, p=0.03) as well as cytosolic calcium levels (AUC 474,587 ± 74,065 $\beta_2$AR desensitized vs 183,564 ± 43,361 non-$\beta_2$AR desensitized, p=0.007) induced by EPN in $\beta_2$AR desensitized HASM cells.

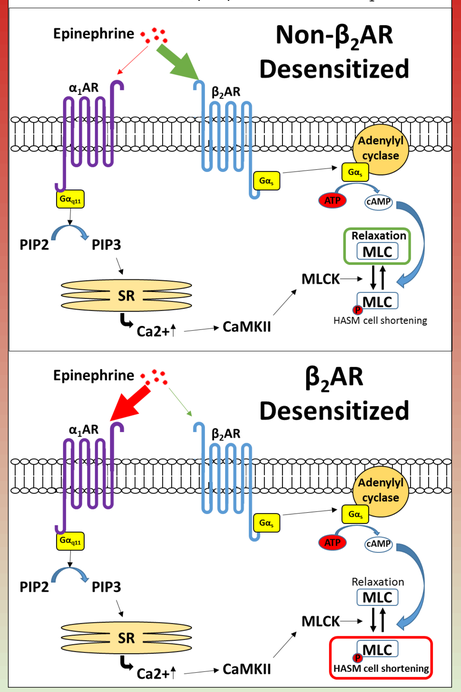
**Conclusions**: These findings suggest that EPN evokes airway smooth muscle cell shortening after $\beta_2$AR desensitization in an $\alpha_1$AR dependent manner.

## Hypothesis

Epinephrine binds the $\alpha_1$AR in HASM cells after $\beta_2$AR desensitization, and evokes smooth muscle shortening instead of relaxation.

## Mechanisms governing adrenergic receptor signaling

Epinephrine binds preferentially to the $\beta_2$AR, but under $\beta_2$AR desensitization, epinephrine binds to the $\alpha_1$AR.




## Bronchial tissue expresses $\alpha_1$AR on HASM

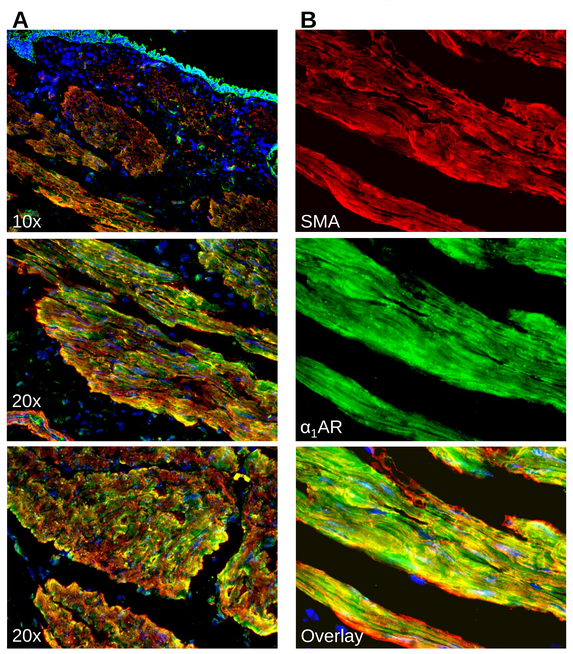



Figure 1. Immunofluorescent staining of (A) human bronchus and (B) human tracheal tissue (40x). Mouse anti-SMA = red, and rabbit anti-$\alpha_1$AR = green.

## MLC phosphorylation increases in response to EPN stimulation under $\beta_2$AR desensitization

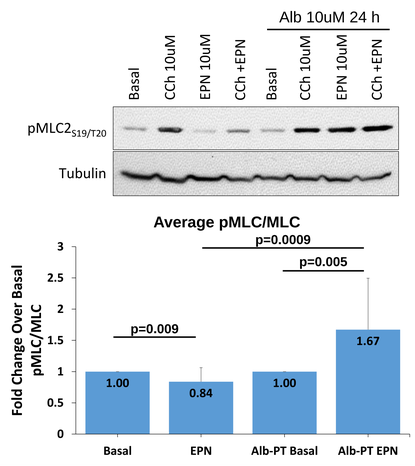



Figure 2. Carbachol (CCh) and Epinephrine (EPN) stimulation without $\beta_2$AR desensitization or after $\beta_2$AR desensitization through twenty-four hour Albuterol pretreatment (Alb-PT). (Mean ± SD, n=14 experiments from 4 unique cell lines)

## EPN increases cytosolic calcium after $\beta_2$AR desensitization

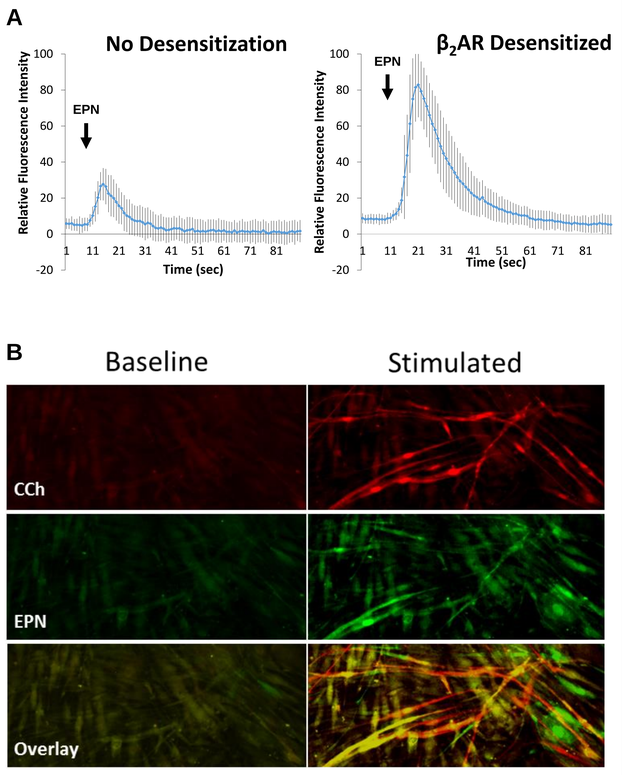



Figure 3. (A) EPN increases cytosolic calcium levels after $\beta_2$AR desensitization in HASM cells (Representative graph is Mean ± SD). (B) $\beta_2$AR desensitized HASM cell calcium responses to CCh (red) and EPN (green) stimulations (25μM). Left panels = baseline fluorescence before stimulation, right panels = 15 sec after stimulation.

## Inhibition of $\alpha_1$AR abrogates pMLC after $\beta_2$AR desensitization

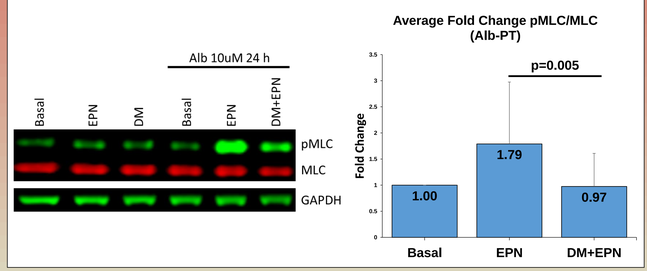



Figure 4. (Left) Representative immunoblot of HASM cells pretreated with 10μM Doxazosin mesylate (DM, $\alpha_1$AR inhibitor) 10 min prior to 10μM EPN stimulation. (Right) Densitometry measured by fold change relative fluorescence units normalized to total MLC relative to Alb-PT basal. (Mean ± SEM, n=5 unique cell lines)

## Inhibition of $\alpha_1$AR decreases EPN induced cytosolic calcium levels after $\beta_2$AR desensitization

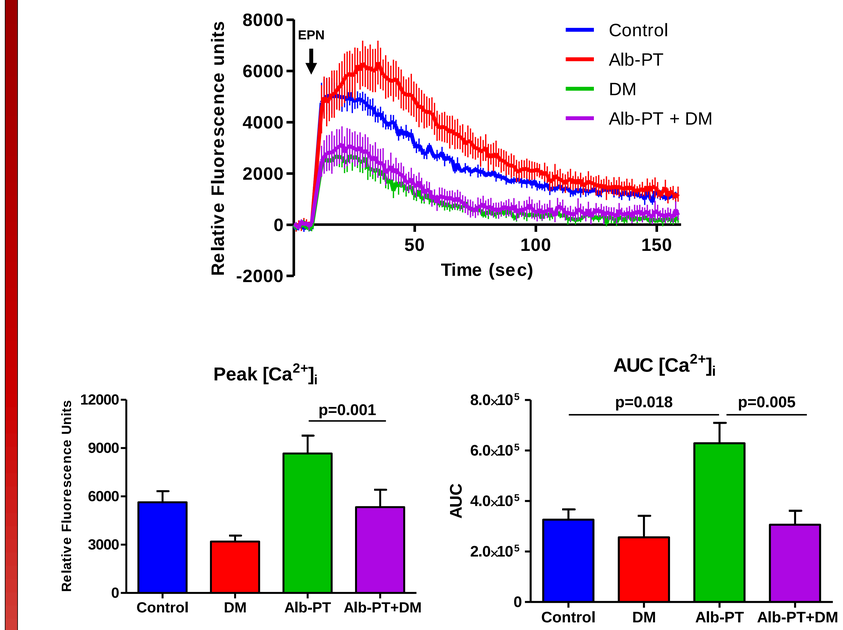



Figure 5. Inhibition of $\alpha_1$AR (DM 1μM) 10min prior to EPN (25μM) stimulation decreases cytosolic calcium levels ($[Ca^{2+}]_i$) as measured by peak relative fluorescence units and area under the curve (AUC) after $\beta_2$AR desensitization. (Mean ± SEM, n=3 unique cell lines run in triplicate)

## Summary

- HASM cells express $\alpha_1$ adrenergic receptor.
- EPN induces increased MLC phosphorylation and cytosolic calcium levels after $\beta_2$AR desensitization.
- Inhibition of $\alpha_1$AR abrogates MLC phosphorylation and cytosolic calcium levels after $\beta_2$AR desensitization of HASM cells.

## Significance

α and β adrenergic receptors modulate responses on the human airway smooth muscle cells and may lead to differential responses to catecholamines. Our data represents a new target for therapeutics aimed at blocking the $\alpha_1$AR to prevent further exacerbations caused by catecholamine release during the stress of an asthmatic event for individuals who do not respond well to $\beta_2$ agonists.

## Acknowledgments

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