Diazepam relaxes mouse peripheral airways in a non-$GABA_A$-receptor dependent manner

Julie Hwang, Jose F Perez-Zoghbi, Gene T Yocum, Maya Mikami, Charles W Emala

Department of Anesthesiology, Vagelos College of Physicians and Surgeons, Columbia University

BACKGROUND

- Benzodiazepines are widely used for sedation and anxiolysis in various settings
- Their mechanism via $GABA_A$ receptor ($GABA_AR$) modulation in the CNS is well known, but their direct effects on peripheral airways are poorly understood
- Our lab has previously published that a novel synthetic benzodiazepine called PI320 can relax mouse peripheral airways by inhibiting calcium mobilization[1]
- In this study, we looked at the ability of clinically used benzodiazepines to relax constricted mouse peripheral airways using precision-cut ling slices
- We further investigated the cellular mechanism underlying benzodiazepine-induced airway relaxation
- Previous studies in guinea pig cardiac tissue and human embryonic kidney (HEK) 293 cells show that diazepam can inhibit cyclic AMP (cAMP)–specific phosphodiesterase type 4 (PDE4)[2-3]

HYPOTHESIS

Clinically used benzodiazepines such as diazepam can relax mouse peripheral airways by a mechanism inhibiting PDE4, independent of the canonical $GABA_AR$ pathway.

METHODS

Precision-Cut Lung Slice (PCLS) Preparation

- Lungs were isolated from C57BL/6J mice and airways inflated via the trachea with warm agarose; pulmonary vasculatures were perfused with warm gelatin to preserve tissue architecture
- Tissue cores were sectioned into ~300 µm slices using a vibratome and incubated in DMEM/F-12 at 37°C, 5% CO_2 until agarose washout was complete

Perfusion Setup

- PCLS were mounted in a perfusion chamber on an inverted phase-contrast microscope and continuously superfused with HBSS ± drug treatment at 37°C
- *Contraction:* Airways were pre-contracted with methacholine (MCh), a synthetic derivative of acetylcholine that induces bronchoconstriction via muscarinic receptors
- *Relaxation:* Following maximal MCh contraction, slices were perfused with diazepam, lorazepam, and midazolam at varying doses to assess bronchodilatory response and generate an IC50 curve

Image Analysis

- Airway lumen area was traced in ImageJ and normalized to the baseline to quantify airway constriction and relaxation

Cyclic AMP ELISA Assay

- Immortalized human airway smooth muscle (hASM) cells were used to measure cAMP levels via ENZO cAMP ELISA assay kit, per the manufacturer's guidelines

RESULTS

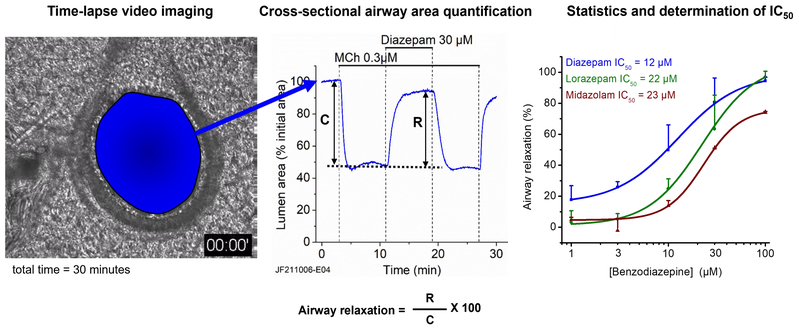

Figure 1. Clinically used benzodiazepines such as diazepam, lorazepam, and midazolam can robustly and reversibly relax MCh-constricted mouse peripheral airways.

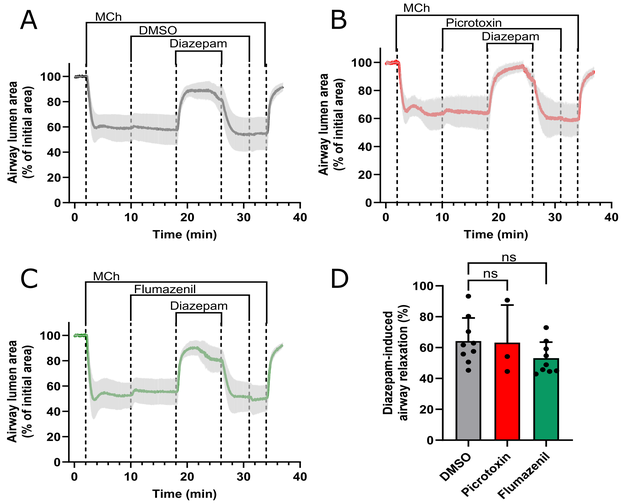

Figure 2. $GABA_AR$ antagonists such as flumazenil or picrotoxin do not block diazepam-induced airway relaxation in mouse PCLS.

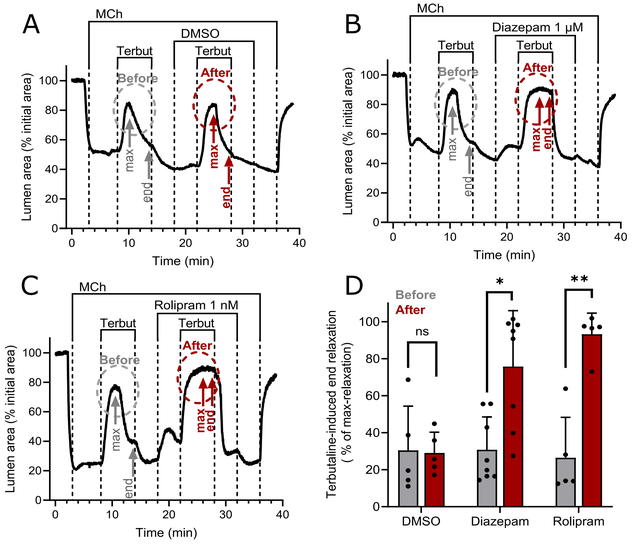

Figure 3. Both diazepam and rolipram (a known PDE4 inhibitor) prolong peripheral airway relaxation induced by the the β2-AR agonist, terbutaline .

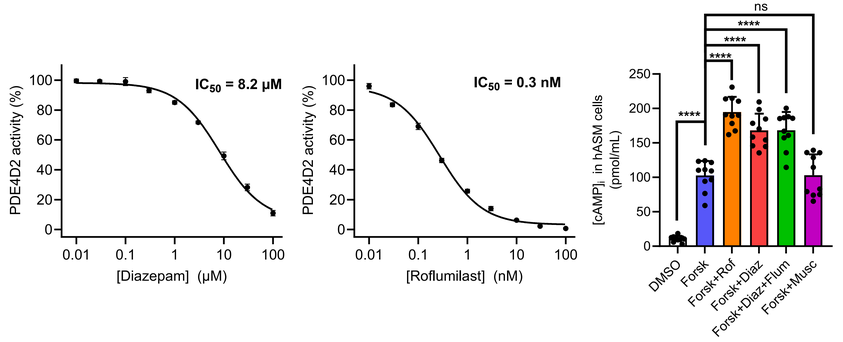

Figure 4. Diazepam directly inhibits purified phosphodiesterase enzyme (PDE4D2) activity and elevates CAMP levels in cultured human ASMCs comparably to roflumilast, a known PDE4 inhibitor.

RESULTS

- Clinically used benzodiazepines (diazepam, lorazepam, and midazolam) robustly and reversibly relaxed MCh-constricted mouse peripheral airways in PCLS (Fig. 1). Diazepam was slightly more potent and thus selected in the subsequent mechanistic studies.
- Pre-treatment with the competitive and non-competitive $GABA_AR$ antagonists, flumazenil and picrotoxin respectively, did not inhibit diazepam-induced airway relaxation, indicating this bronchodilatory effect is independent of the $GABA_AR$ pathway (Fig. 2).
- Diazepam prolonged terbutaline-induced peripheral airway relaxation, as did rolipram, a known PDE4 inhibitor (Fig. 3).
- Diazepam directly inhibited PDE4D2 activity in a purified enzyme assay, and elevated cAMP levels in cultured hASM cells even in the presence of flumazenil (Fig. 4). Muscimol, a potent $GABA_AR$ agonist, did not raise cAMP levels in hASM.

DISCUSSION

- Clinically used benzodiazepines robustly and reversibly relax precontracted mouse peripheral airways
- Diazepam inhibits PDE4 activity to increase cAMP levels in airway smooth muscle cells
- This PKA-mediated pathway is independent of the $GABA_AR$ mechanism associated with benzodiazepines
- The synergistic interaction between diazepam and β2-AR agonists may have significant clinical implications for airway management in high-risk patients
- Diazepam may be administered as an adjunct alongside β2-AR agonists such as albuterol to provide dual benefits of anxiolysis and potentiation of the bronchodilatory effects of β2-AR agonists

Limitations & Future Directions:

- Studies were conducted in mouse instead of human lungs
- Expand mechanistic studies to include other clinically used benzodiazepines such as midazolam, which would be more relevant in the intraoperative setting

REFERENCES

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No Conflicts of Interest