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Evaluation of lysosomal sequestration in bronchial epithelial cells as a mechanism of drug retention in the lungs

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Major challenges in the development of inhaled drugs

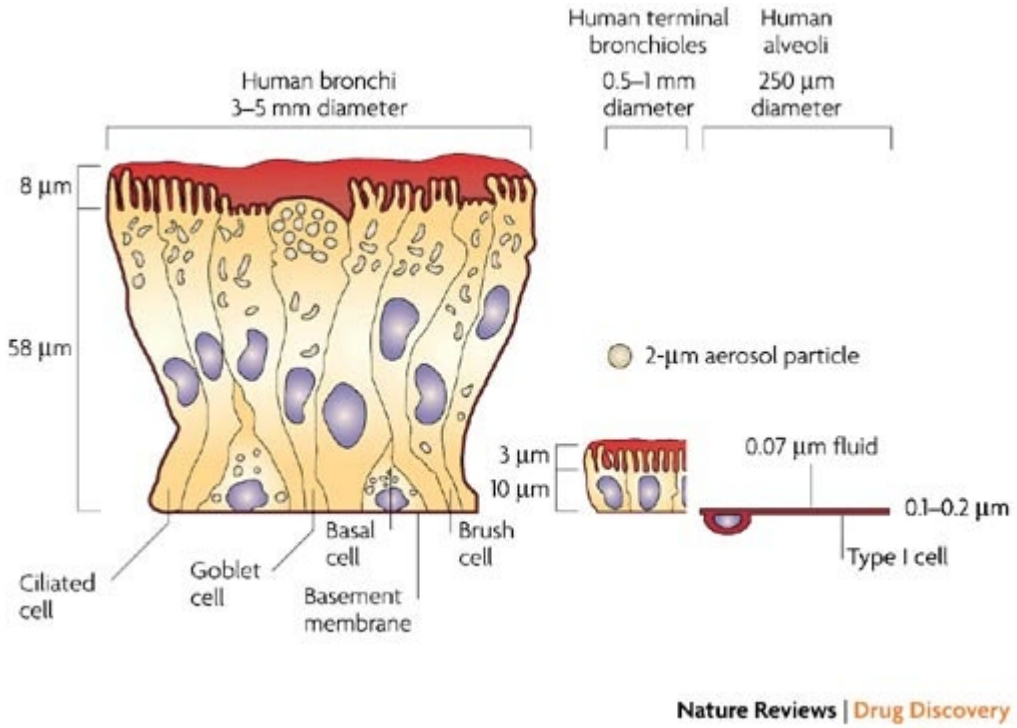
- Rapid absorption into the bloodstream/poor retention in the lung

  → Very high attrition rate during pre-clinical development

- Strong reliance on in vivo studies in laboratory animals

  → Mechanisms governing drug disposition in the lungs remain poorly understood

The airway epithelium is the first barrier encountered by inhaled drugs



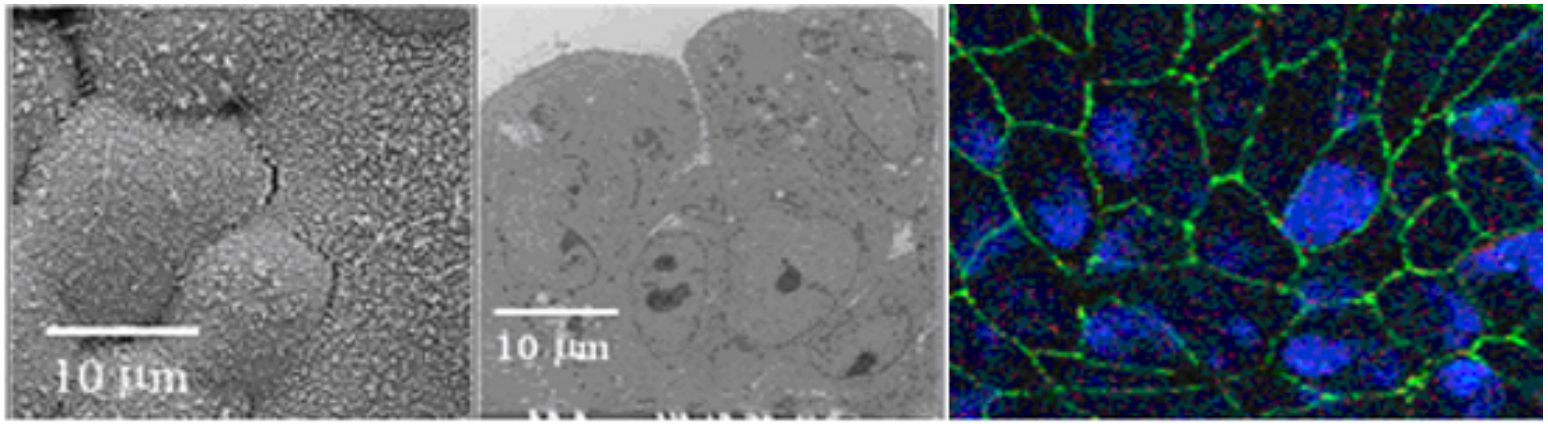
- **Does it play a role in controlling drug retention in the lungs?**
- **What are the mechanisms involved?**

→ **In vitro models needed to answer those questions**

Schematic from Patton & Byron, Nature Review Drug Discovery, 2007

Calu-3 layers mimic the native bronchial epithelium

- Human broncho-epithelial cancerous cell line
- Mixed population of ciliated and goblet cells
- Forms differentiated cell layers when grown at an air-liquid interface (ALI)
- Transepithelial electrical resistance (TEER) > 400 ohm.cm^2
- Express the range of drug transporters present in the lung when grown at an ALI for 21 days*



Grainger C et al, Pharm Res, 2006
* Hutter et al, Eur.J.Pharm.Biopharm, 2014

Aim of the study

Can the Calu-3 model help understanding the mechanisms of drug retention in the lung tissue?

Hypothesis

Sequestration in the lysosomes of airway epithelial cells may play an important role*

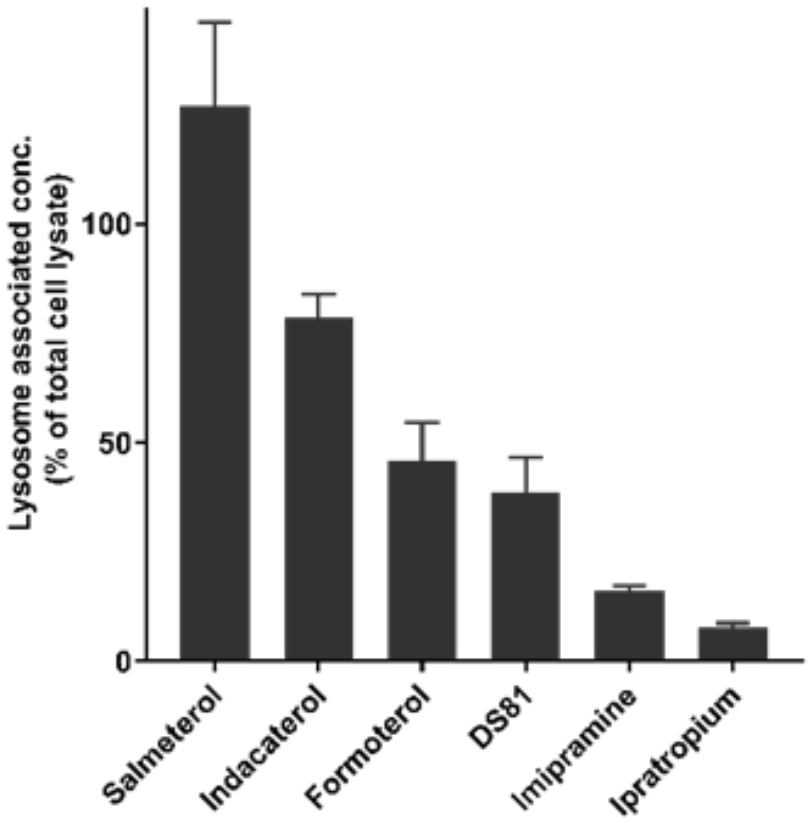
*Bäckström et al, J.Pharm.Sci, 2016

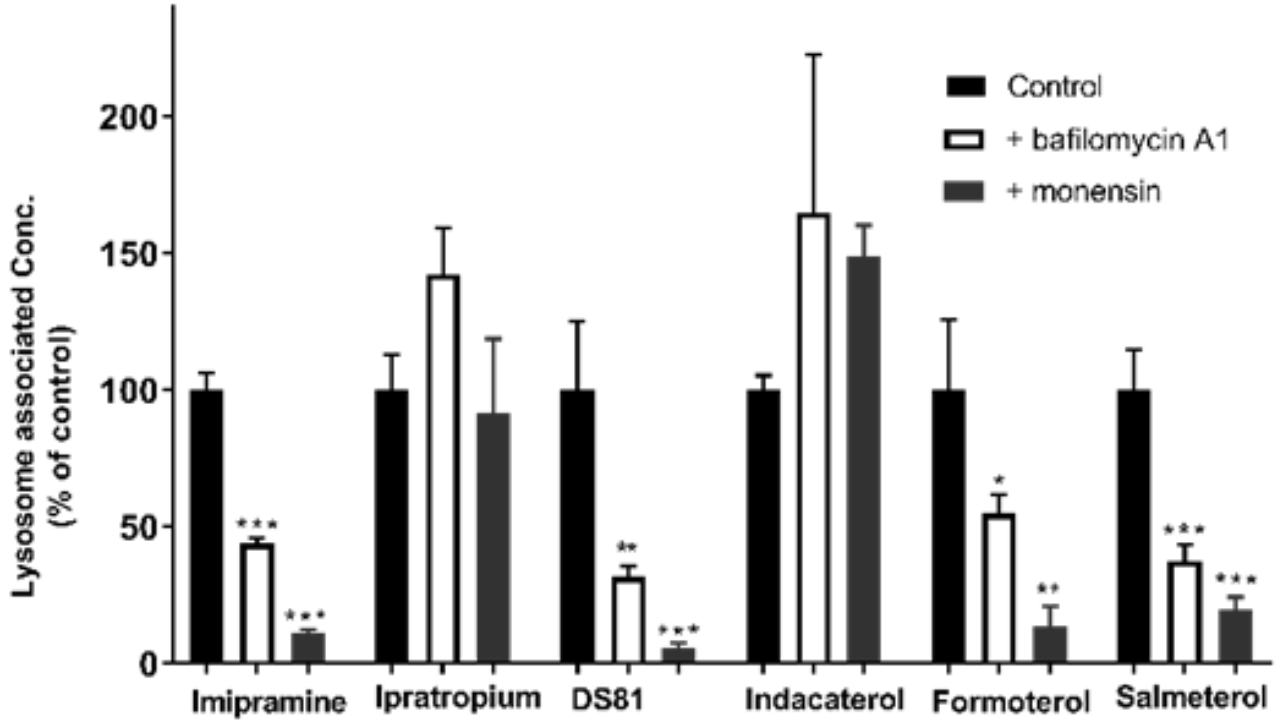
Compounds tested in the study

Compound	Class	Structure	Duration of action	LogP
Salmeterol	B_2-agonist	monobase	long	3.73
Indacaterol	B_2-agonist	zwitterion	long	4.27
Formoterol	B_2-agonist	monobase	long	2.03
Salbutamol	B_2-agonist	monobase	short	0.61
Ipratropium	M3 antagonist	quaternary	short	-1.8
DS81	Investigational	dibase	unknown*	3.56

*$T_{1/2}$ in lung tissue: 19h

Quantification of lysosomal concentrations after sub-cellular fractionation

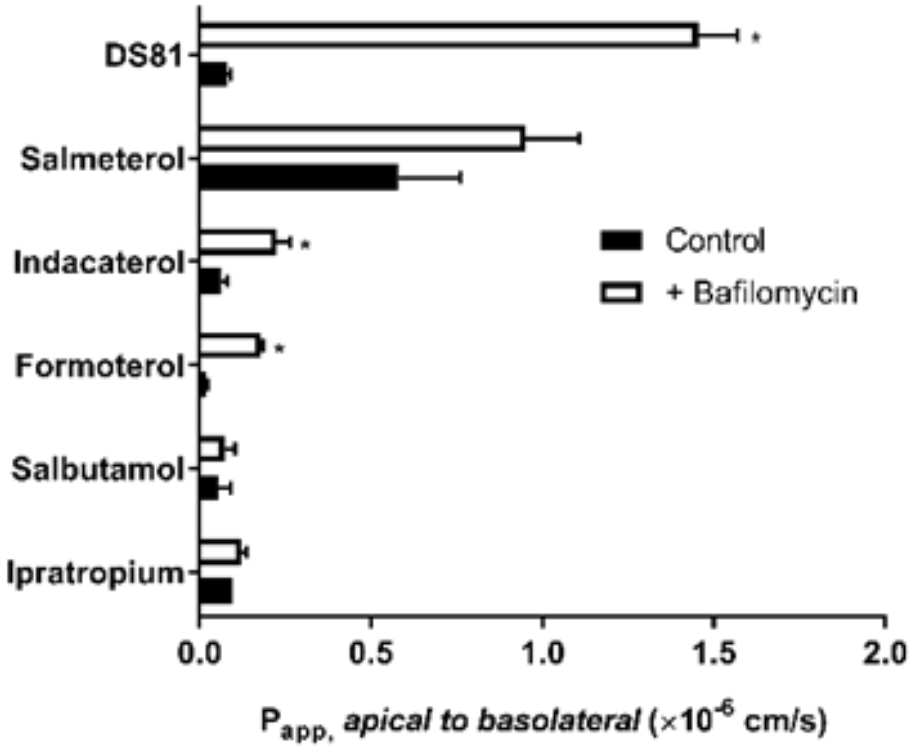


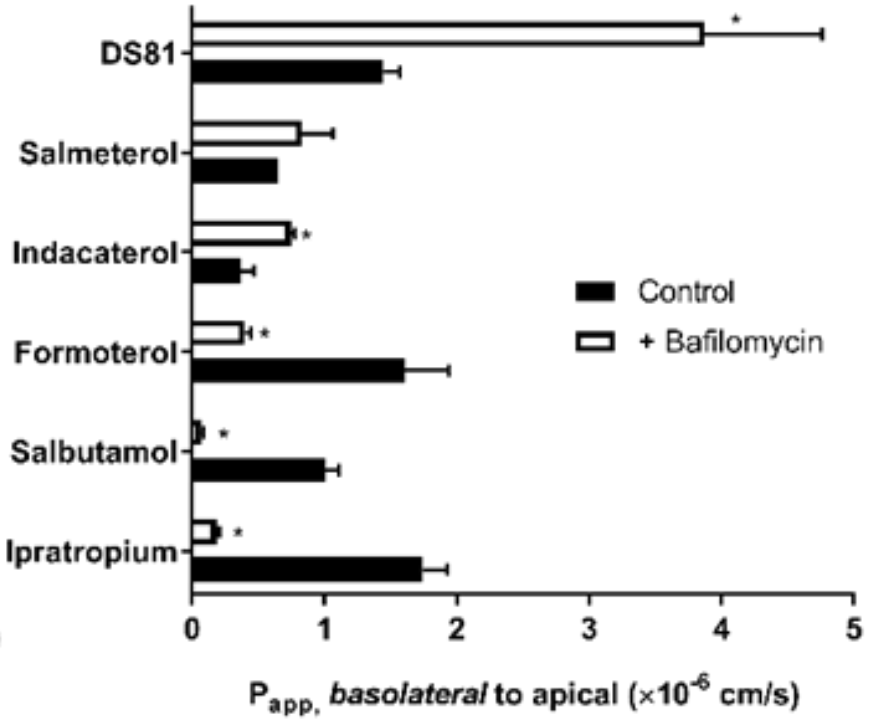


Data are mean ± SD (n=3-4)

- Significant lysosomal sequestration of lipophilic compounds
- No effect of inhibitors on ipratropium and indacaterol sequestration

Bidirectional permeability studies in ALI Calu-3 layers



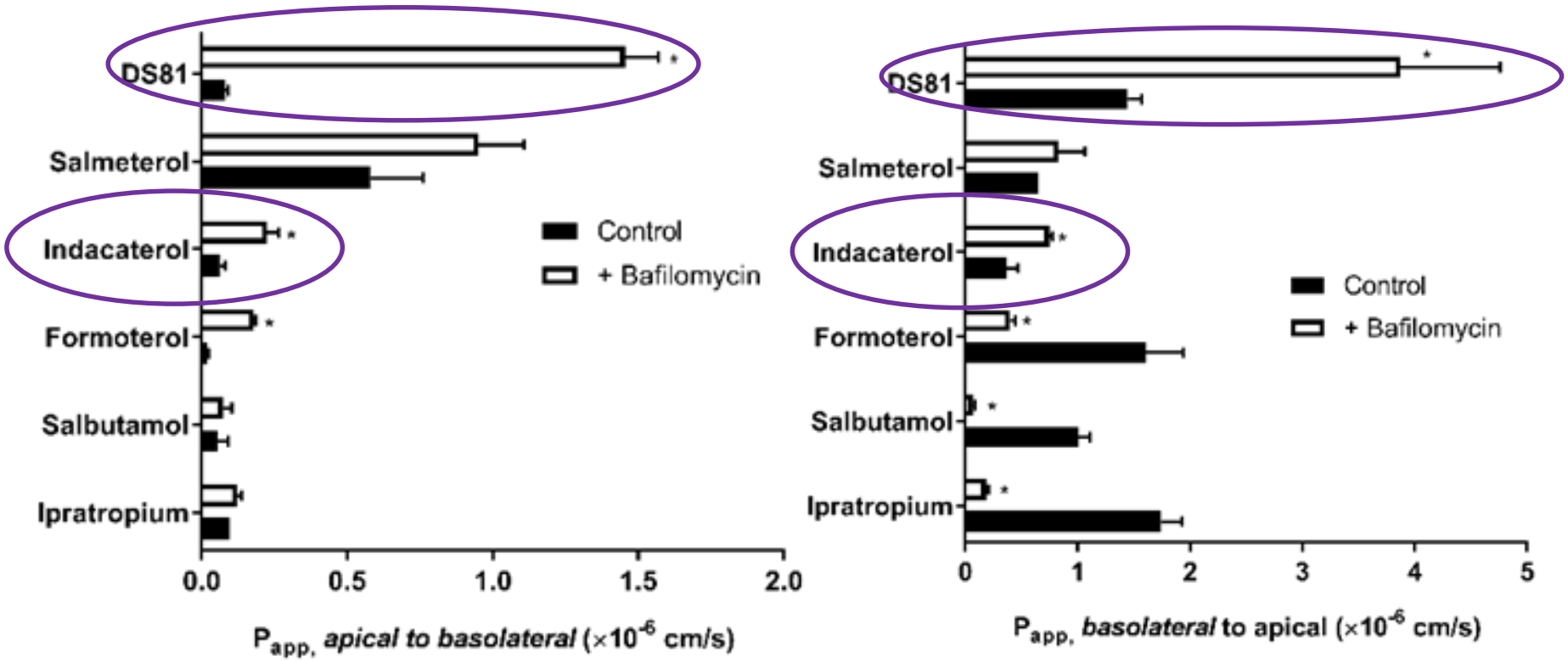


Efflux Ratio
18.3 ± 1.5
1.1 ± 0.0
5.8 ± 1.6
> 20
18.5 ± 1.8
17.7 ± 1.9

Data are mean ± SD (n=3-4)

Apparent efflux for all compounds but salmeterol

Bidirectional permeability studies in ALI Calu-3 layers

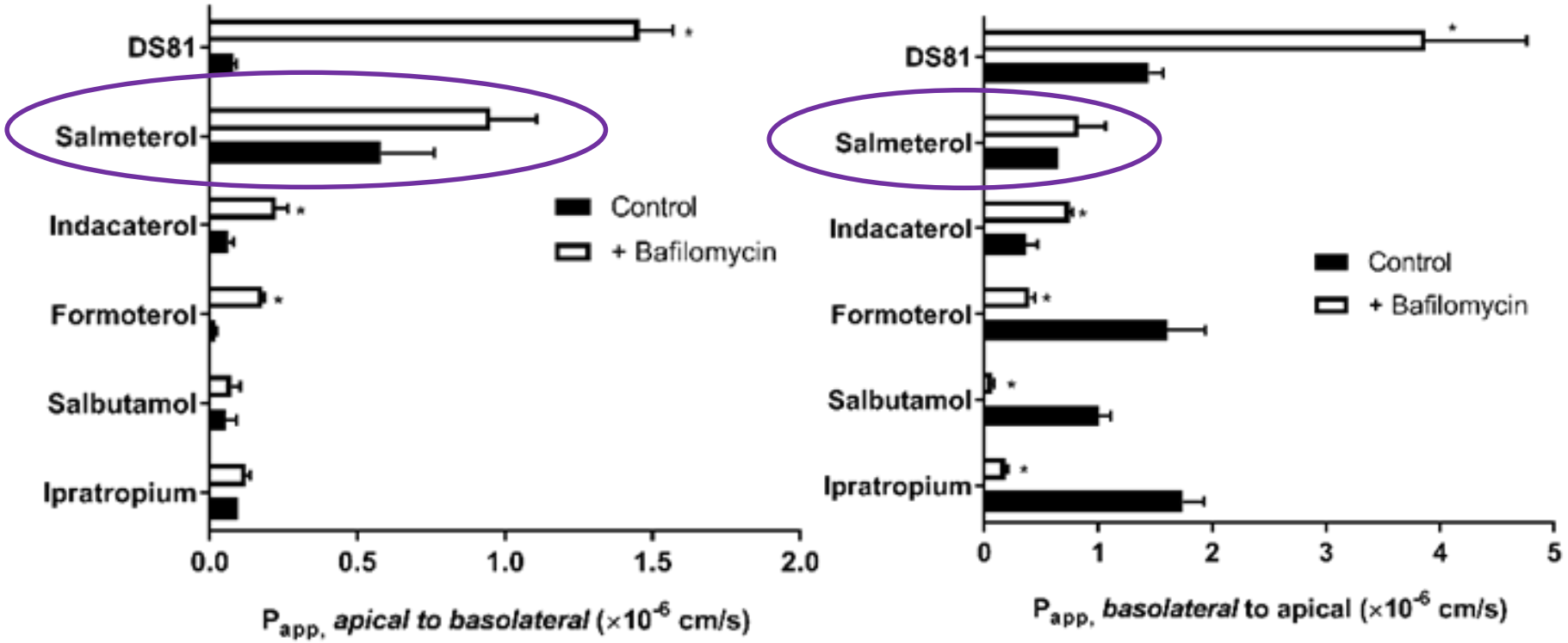


Data are mean ± SD (n=3-4)

Increase in permeability in both directions with bafilomycin

→ Lysosomal trapping partly explains very low absorption?

Bidirectional permeability studies in ALI Calu-3 layers

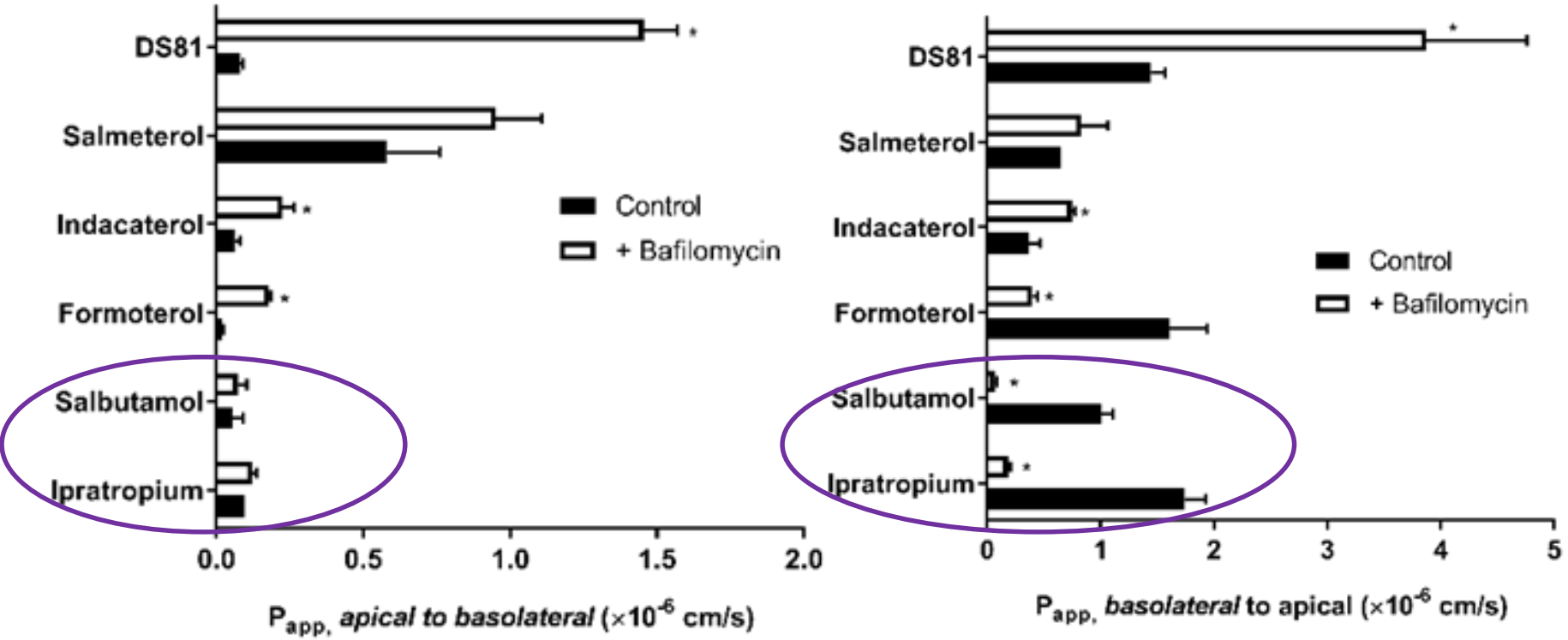


Data are mean ± SD (n=3-4)

No effect of bafilomycin

→ Passive diffusion overcome lysosomal sequestration?

Bidirectional permeability studies in ALI Calu-3 layers



Data are mean ± SD (n=3-4)

No effect of bafilomycin in absorptive direction; decreased secretory permeability → Interference with a basolateral transporter?*

Guckel et al, Cell.Physiol.Biochem., 2012

Conclusions

- Lysosomal trapping in bronchial epithelial cells is likely to determine the lung retention of basic/dibasic inhaled drugs
- All compounds investigated but salmeterol were subjected to an efflux system in Calu-3 cell layers
- Insight into drug disposition mechanisms in the lung can be obtained using bronchial epithelial cell layers in vitro.
- Larger studies are required to increase confidence in the models

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