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Bronchoconstriction in nonhuman primates: a species comparison

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Seehase S, Schlepütz M, Switalla S, Mätz-Rensing K, Kaup FJ, Zöller M, Schlumbohm C, Fuchs E, Lauenstein HD, Winkler C, Kuehl AR, Uhlig S, Braun A, Sewald K, Martin C. Bronchoconstriction in nonhuman primates: a species comparison. *J Appl Physiol* 111: 791–798, 2011. First published June 23, 2011; doi:10.1152/jappphysiol.00162.2011.—Bronchoconstriction is a characteristic symptom of various chronic obstructive respiratory diseases such as chronic obstructive pulmonary disease and asthma. Precision-cut lung slices (PCLS) are a suitable ex vivo model to study physiological mechanisms of bronchoconstriction in different species. In the present study, we established an ex vivo model of bronchoconstriction in nonhuman primates (NHPs). PCLS prepared from common marmosets, cynomolgus macaques, rhesus macaques, and anubis baboons were stimulated with increasing concentrations of representative bronchoconstrictors: methacholine, histamine, serotonin, leukotriene D₄ (LTD₄), U46619, and endothelin-1. Alterations in the airway caliber were measured and compared with previously published data from rodents, guinea pigs, and humans. Methacholine induced maximal airway constriction, varying between 74 and 88% in all NHP species, whereas serotonin was ineffective. Histamine induced maximal bronchoconstriction of 77 to 90% in rhesus macaques, cynomolgus macaques, and baboons and a lesser constriction of 53% in marmosets. LTD₄ was ineffective in marmosets and rhesus macaques but induced a maximum constriction of 44 to 49% in cynomolgus macaques and baboons. U46619 and endothelin-1 caused airway constriction in all NHP species, with maximum constrictions of 65 to 91% and 70 to 81%, respectively. In conclusion, PCLS from NHPs represent a valuable ex vivo model for studying bronchoconstriction. All NHPs respond to mediators relevant to human airway disorders such as methacholine, histamine, U46619, and endothelin-1 and are insensitive to the rodent mast cell product serotonin. Only PCLS from cynomolgus macaques and baboons, however, responded also to leukotrienes, suggesting that among all compared species, these two NHPs resemble the human airway mechanisms best.

baboon; lung explants; macaque; marmoset; precision-cut lung slices

OBSTRUCTIVE LUNG DISEASES such as asthma and chronic obstructive pulmonary disease are characterized by a number of distinct symptoms including acute bronchospasm, inflammation, and mucus hypersecretion (9). Increased sensitivity of the airways results in airway hyperresponsiveness, facilitating constriction of bronchial smooth muscles in response to a variety of stimuli. This fact is made use of in diagnosing bronchial

hyperreactivity and in evaluating the efficacy of therapeutic agents in human clinical trials, where provocations with methacholine or histamine are frequently utilized (35). Other clinical bronchoconstrictors are cysteinyl leukotrienes and thromboxane (4), and also endothelin-1, which play an important role in acute and chronic lung inflammation including pneumonia and asthma (18).

There is a growing burden of obstructive lung diseases, and currently available treatments have only minimal impact on disease progression (16). Thus development of new therapeutics is needed to alleviate or even cure symptoms of respiratory obstructive diseases (3). At present, rodents are the most commonly used species in animal models of obstructive lung diseases (3, 7). Rodent models, however, are often limited in reflecting the human situation because of anatomical, physiological, and immunological differences. For example, there are differences in architecture and cellular composition of the tracheobronchial airways, such as the monopodial branching pattern in rodents and the dichotomous branching pattern in humans and nonhuman primates (NHPs; Ref. 42). Other animal models for studying fundamental immunological causes of asthma and for preclinical testing of pharmaceuticals have been established in dogs (39), sheep (1, 48), and NHPs (6, 21, 46, 55, 61). NHPs emerge as one of the most suitable animal models to reflect the human situation and may be the model of choice for the human lung. They share various anatomical similarities and have a high homology to different human target structures because of their close phylogenetic relation (14). The Old World monkeys cynomolgus macaque (*Macaca fascicularis*), rhesus macaque (*Macaca mulatta*), and anubis baboon (*Papio anubis*) as well as the New World monkey common marmoset (*Callithrix jacchus*) are used in biomedical research. So far, several respiratory in vivo studies have been performed in *Ascaris suum* or house dust mite-sensitized cynomolgus and rhesus macaques (6, 21, 46, 55, 61) and in nonsensitized baboons (5, 44, 57, 60). In most of these in vivo studies, lung function was measured by noninvasive forced oscillation techniques (6, 52), which are also used in young children and adults (37, 53).

The use of NHPs in research, however, is often viewed critically due to ethical concerns. In line with Russell's and Burch's "3 Rs" concept (replacement, reduction, and refinement), several alternative methods to reduce pain, distress, and damage to laboratory animals were established for the investigation of basic mechanisms of lung physiology. Various ex vivo techniques have been developed to assess airway function in different species (17, 45, 54). Precision-cut lung slices (PCLS) display a convenient ex vivo model that offers an intact

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microanatomy of the lung and represent a suitable alternative to in vivo studies regarding cytokine secretion and airway function measurements (24). Furthermore, PCLS allow the investigation of pharmacological effects under identical experimental conditions in different species and require only small numbers of animals. So far, the PCLS technique has been successfully used to study bronchoconstriction in lung tissue from rodents (23, 32), guinea pigs (43), horses (56), humans (43, 58), and rhesus macaques (25, 26, 28). The exact physiological mechanisms of bronchoconstriction in NHP were, however, not previously investigated.

In the present study, we established the technique of PCLS in NHPs to analyze airway responsiveness to various bronchoconstrictors via videomicroscopy. In particular, we studied three Old World primate species, anubis baboon, rhesus macaque, and cynomolgus macaque, and one New World primate species, common marmoset. The aim of this study was to compare physiological airway responsiveness in different NHPs to identify the NHP that is most relevant for the situation in humans. We further accomplished an interspecies comparison among NHPs, humans, and common laboratory animals such as guinea pigs and rodents.

MATERIAL AND METHODS

Animals. Lungs from different adult NHP species were obtained from the German Primate Center (Goettingen, Germany), with the exception of three female cynomolgus macaque lungs, which were obtained from Covance Laboratories (Muenster, Germany). The NHPs used for this study included four cynomolgus macaques (*Macaca fascicularis*), five rhesus macaques (*Macaca mulatta*), four anubis baboons (*Papio anubis*), and six common marmosets (*Callithrix jacchus*) (Table 1). All animals were euthanized with an anesthetic overdose (iv) according to the EU Guideline 2010/63/EU. Old World monkeys were euthanized with pentobarbital sodium, whereas ketamin was used for euthanasia of common marmosets. At the time of necropsy, the animals did not show any remarkable clinical symp-

toms indicative of pathological changes in the respiratory tract. All animals were part of control groups and were not treated with any substances.

Care and housing conditions of the animals complied with the regulations of the European Parliament and the Council Directive on the protection of animals used for scientific purposes (2010/63/EU) and the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*. This includes an ethical approval of the responsible animal welfare officer of the German Primate Center and authorization by the regional governmental veterinary authorities (Goettingen, Lower Saxony, Germany).

Media, reagents, and chemicals. PBS (0.1 M sodium phosphate and 0.15 M NaCl, without Ca^{2+} and Mg^{2+}) was obtained from Lonza (Verviers, Belgium). Dulbecco's modified Eagle's medium Nutrient Mixture F-12 Ham (DMEM) with L-glutamine and 15 mM HEPES without phenol red, pH 7.2–7.4, was supplied by Sigma-Aldrich (Munich, Germany) and supplemented with 7.5% w/v sodium bicarbonate. MEM (pH 7.2), composed of CaCl_2 (1.8 mM), MgSO_4 (0.8 mM), KCl (5.4 mM), NaCl (116.4 mM), glucose (16.7 mM), NaHCO_3 (26.1 mM), HEPES (25.17 mM), sodium pyruvate, amino acids, vitamins, and glutamine was supplemented with 1% (vol/vol) penicillin and streptomycin (all from PAA Laboratories). Triton X-100, 10% neutral-buffered formalin, and low-gelling agarose were purchased from Sigma-Aldrich.

Preparation of NHP PCLS. NHP PCLS were prepared as previously described for other species (32, 58). Briefly, lungs were removed from healthy adult monkeys, which were euthanized for other purposes, and filled up with warm (37°C) liquid low-gelling agarose [1.5% (wt/vol)]/DMEM. The agarose-filled tissue was allowed to solidify on ice. In case of the marmosets, whole lungs were filled via the trachea and lobes were separated after polymerization of the agarose. In Old World monkeys, only the left inferior lobes were filled via the main bronchus. The lung lobes were cut into ~20-mm blocks plane perpendicular to the long axis. Tissue cores with a centered airway were prepared with a rotating sharpened metal tube (diameter: 10 mm), and 220- μm thin tissue slices were cut perpendicular to the airway with a microtome (Krumdieck tissue slicer; Alabama Research and Development, Munford, AL). Selection of PCLS was based on similar airway size varying from 0.3 to 1 mm. Approximately 30 PCLS per animal were performed. PCLS were incubated at 37°C in a humid 5% carbon dioxide atmosphere under normal cell culture conditions in MEM. Medium was changed every 30 min during the first 2 h after preparation and every hour for the next 2 h to remove cell debris from the tissue. For each mediator, two PCLS in average were used.

Preparation and data mining of human PCLS. The human PCLS experiments and data refer to the study performed by Ressmeyer et al. (43). In this study, human lung material was obtained from patients undergoing lobectomy due to cancer. After pathological inspection, cancer-free tissue was used. The experiments were approved by the local ethics committee, and all patients gave written informed consent.

Lung lobes were filled via the main bronchus with a 1.5% low-gelling agarose-medium solution and were solidified on ice. Then, tissue preparation, i.e., coring slicing and culturing, followed as described for NHP above.

Cumulative concentration-response curves for methacholine, histamine, serotonin, leukotriene D_4 (LTD_4), and U46619 were conducted on human PCLS from four to five patients in total and were performed the same way as in NHPs (see below).

Viability assays. Tissue viability before bronchoconstriction was proven by measuring the release of lactate dehydrogenase (LDH) into the incubation medium (43). LDH activity was determined in culture supernatant using a commercial enzymatic assay (Roche, Mannheim, Germany). PCLS, permeabilized with Triton X-100 (1% in PBS), were used as reference (100% dead cells). After incubation according to the manufacturer's instructions, absorbance was determined at 450 nm with a reference wave length of 690 nm.

Table 1. History of the animals used in the present study

Species/Animal No.	Sex	Weight, kg	Age, yr
Cynomolgus macaque			
1	Female	3.50	4.6
2	Female	3.00	5.8
3	Female	5.00	8.4
4	Male	6.00	7.1
Rhesus macaque			
5	Male	10.20	5.9
6	Male	3.00	3.5
7	Female	2.80	1.8
8	Female	6.30	6.6
9	Female	4.50	18.6
Baboon			
10	Male	20.00	12.4
11	Female	12.80	11.5
12	Female	12.30	17.1
13	Female	13.00	4.5
Marmoset			
14	Male	0.22	2.0
15	Male	0.30	6.3
16	Female	0.43	3.1
17	Male	0.36	5.9
18	Female	0.34	3.2
19	Female	0.34	7.1

None of the animals had previously been used in studies involving the respiratory system or manipulation of the immune system or drug testing.

Tissue viability after bronchoconstriction measurement was proven by colorimetric assays (50): WST-1 (Roche, Mannheim, Germany) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich). For WST-1, tissue was incubated for 1 h at 37°C with 0.125 ml WST-1 solution (diluted 1:10 in freshly prepared culture medium) according to the manufacturer's instructions. Absorbance of the formazan solution in the supernatant was determined at 450 nm with a reference wave length of 690 nm. For the MTT test, PCLS were incubated for 15 min at room temperature in 1 ml MEM with 0.7 mg/ml MTT. Supernatants were removed, and 200 μ l of formic acid/propanol (5%/95%) solution were added to each slice. After incubation for 20 min, 100 μ l of supernatant were used to measure the absorbance at 550 nm.

After bronchoconstriction measurements, the agarose-filled lung tissue was fixed in 10% neutral-buffered formalin and embedded in paraffin to evaluate the health status of the lungs histologically. Embedded tissues were sectioned (at 4 μ m), placed on glass slides, and stained with hematoxylin and eosin for subsequent examination by light microscopy.

Measurement and imaging of bronchoconstriction. Peripheral airways were imaged and digitized using an inverted microscope (DMIL Leica, Wetzlar, Germany) and a digital video camera (SensiCam 365KL; Visitron Systems, Munich, Germany). Camera control and image analysis were performed using the Optimas 6.5 software (Optimas, Bothell, WA). The airway area before addition of the lowest concentration of the agonist was defined as 100%. Bronchoconstriction was expressed as percentage of the initial airway area. Only slices with airways free of agarose, with beating cilia, and with an intact smooth muscle layer in a relaxed state were taken into account. The relaxed state was defined as the presence of a smooth inner airway surface ascertain by transmitted light microscopy.

For measurement of bronchoconstriction, each tissue section was placed into one well of a standard 24-well cell culture plate and was fixed by a nylon thread attached by a platinum wire at the bottom of the dish to avoid its moving during measurement. Cumulative concentration-response curves were performed for methacholine (10^{-10} – 10^{-4} M), serotonin (10^{-10} – 10^{-4} M), and histamine (10^{-8} – 10^{-3} μ M; all from Sigma-Aldrich, Munich, Germany); endothelin-1 (10^{-11} – 10^{-6} M; Bachem, Weil, Germany); and LTD₄ (10^{-10} – 10^{-6} M) and U46619 (10^{-11} – 10^{-5} M; both from Cayman Chemicals, Biomol, Hamburg, Germany). Images were recorded for methacholine, histamine, and serotonin every 5 s for a time period of 5 min, for endothelin-1 every 30 s for 10 min, and for LTD₄ and U46619 every 30 s for 20 min until the next concentration was added. The half-maximal responses were calculated by nonlinear regression with a four-parameter logistic equation and are denoted as EC₅₀.

Statistics. The GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA) was used for fitting sigmoidal concentration-response curves and calculation of EC₅₀ values. Maximum constriction (C_{max}) and EC₅₀ values were analyzed using one-way ANOVA and Dunnett's multiple comparison test against human data. Data are expressed as means $\pm$ SE or means $\pm$ SD as indicated.

RESULTS

For all NHPs, PCLS with intermediate sized airways varying from 0.3 to 1 mm were performed as shown in Fig. 1 using the example of cynomolgus macaque PCLS. All used NHP lungs did not show any histopathological abnormalities. PCLS were viable before and after bronchoconstriction measurement as assessed by LDH release and WST-1 or MTT assay (data not shown). None of the mediators used for induction of bronchoconstriction caused cytotoxicity in lung tissue.

Methacholine was able to induce airway constriction in all NHP species (Fig. 2A), with decreasing sensitivity in the following order: baboon (EC₅₀ = 25 nM) > marmoset (EC₅₀ = 43 nM) > rhesus macaque (EC₅₀ = 195 nM) > cynomolgus macaque (EC₅₀ = 718 nM). Compared with previously published human data (43), anubis baboons and common marmosets showed significantly lower EC₅₀ values for methacholine-induced bronchoconstriction, whereas cynomolgus macaques showed significant higher EC₅₀ values (Table 2). All species revealed, however, a maximum bronchoconstriction of $\geq 74\%$ (Fig. 2A; Table 2).

Histamine was also identified as a strong bronchoconstrictor in all NHP PCLS, with decreasing sensitivity in the following order: baboon (EC₅₀ = 244 nM) > marmoset (EC₅₀ = 409 nM) > rhesus macaque (EC₅₀ = 894 nM) > cynomolgus macaque (EC₅₀ = 3,208 nM), whereupon only baboons and marmosets showed significant lower EC₅₀ values compared with humans (Table 2). Maximum constriction was $>77\%$ in all NHPs except for marmosets, which showed a maximum airway constriction of 53% (Fig. 2B; Table 2). However, only baboons revealed differences with regard to maximum constriction compared with humans (Table 2; Ref. 43).

In contrast, responses to serotonin were weak in all NHPs as seen in humans (Table 2; Ref. 43). EC₅₀ values were only calculable for rhesus macaques (EC₅₀ = 544 nM) and baboons

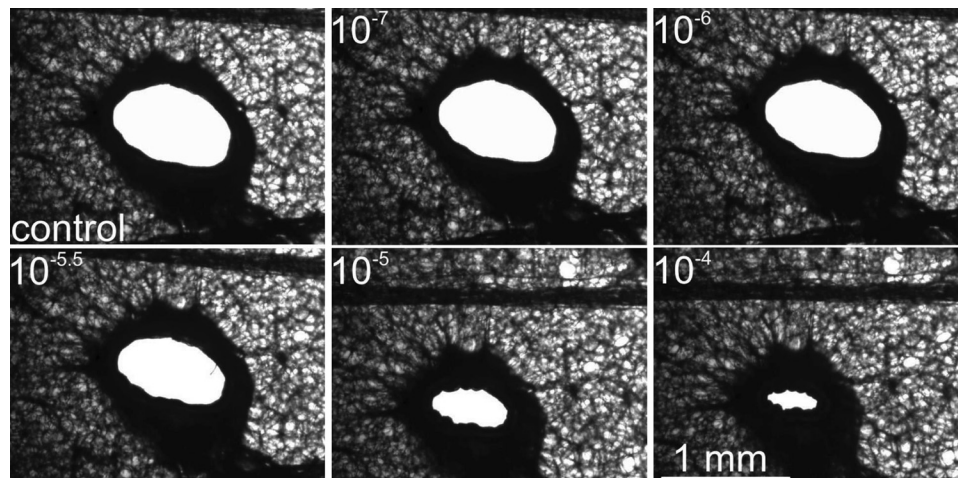


Fig. 1. Histamine-induced bronchoconstriction in precision-cut lung slices (PCLS) of cynomolgus macaque. Histamine was applied to the tissue in concentrations ([M]) as indicated in the left corner of the transmitted light images. Alterations of the airway caliber were determined by using Optimas 6.5 software. Corresponding concentration response curve for histamine-induced bronchoconstriction in cynomolgus macaques is shown in Fig. 2B.

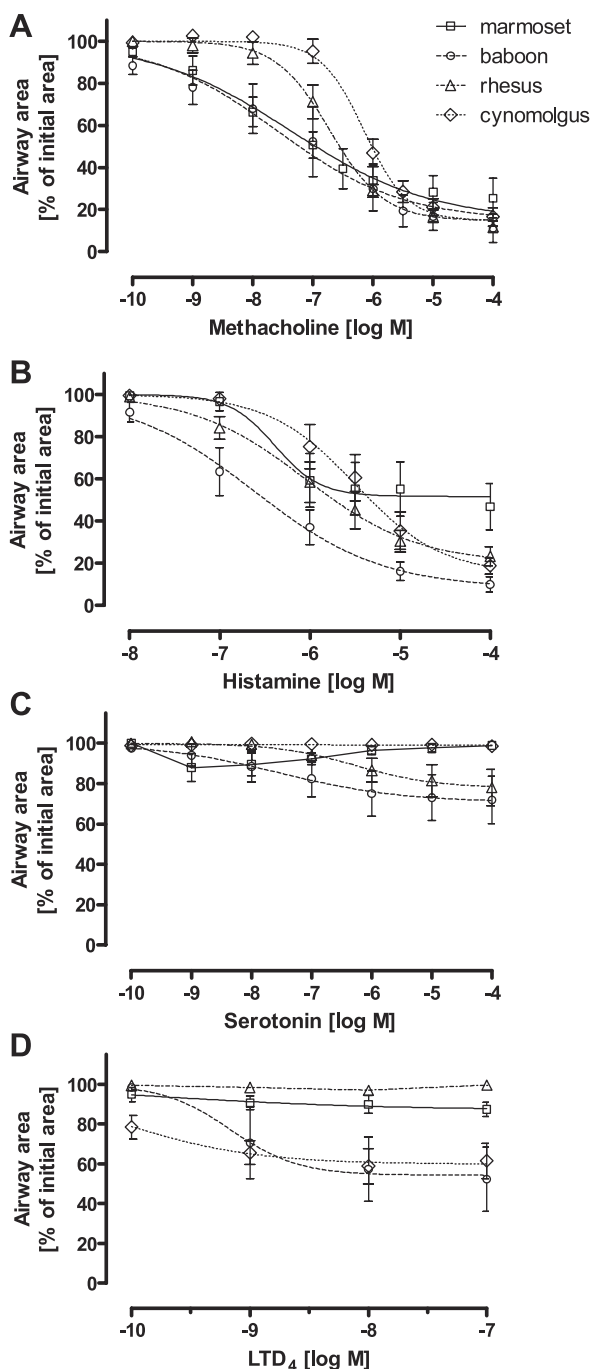


Fig. 2. Effects of methacholine, histamine, serotonin, and leukotriene D₄ (LTD₄) on bronchoconstriction of baboon, marmoset, cynomolgus macaque, and rhesus macaque. Concentration-response curves for the following: A: methacholine [marmoset (□): $n = 6$, half-maximal effective concentration (EC₅₀) = 43 nM; baboon (○): $n = 3$, EC₅₀ = 25 nM; cynomolgus macaque (◇): $n = 5$, EC₅₀ = 718 nM; rhesus macaque (△): $n = 4$, EC₅₀ = 195 nM]; B: histamine [□: $n = 4$, median EC₅₀ = 409 nM; ○: $n = 3$, EC₅₀ = 244 nM; ◇: $n = 5$, EC₅₀ = 3,208 nM; △: $n = 4$, EC₅₀ = 894 nM]; C: serotonin [□: $n = 5$, not responsive; △: $n = 3$, EC₅₀ = 21 nM; ◇: $n = 4$, not responsive; △: $n = 4$, not responsive]; D: LTD₄ [□: $n = 6$, not responsive; ○: $n = 3$, EC₅₀ = 0.7 nM; ◇: $n = 3$, EC₅₀ = 0.3 nM; △: $n = 2$, not responsive]. Data are expressed as means $\pm$ SE.

(EC₅₀ = 29 nM) but not for marmosets or cynomolgus macaques (Fig. 2C; Table 2).

Administration of LTD₄ resulted in a strong airway response in baboons and cynomolgus macaques, with a maximum constriction of 60 and 44%, and EC₅₀ values of 0.7 and 0.09 nM, respectively, being significantly higher than humans (Table 2; Ref. 43). Marmosets and rhesus macaques showed no airway constriction after treatment with up to 0.1 μ M LTD₄ (Fig. 2D).

Effects of the thromboxane prostanoid receptor agonist U46619 and the peptide mediator endothelin-1 were only examined in three NHP species, marmosets, cynomolgus macaques, and baboons. In all NHPs, U46619 induced strong airway constriction, varying between 65% (cynomolgus macaques) and 91% (baboons), not significantly different from humans (Table 2; Ref. 43). The strongest response was detectable in PCLS from baboons (EC₅₀ = 0.03 nM), followed by cynomolgus macaques (EC₅₀ = 0.4 nM) and marmosets (EC₅₀ = 10.7 nM; C_{max} 69%; Fig. 3A; Table 2).

Endothelin-1 elicited strong airway contraction in all three NHPs studied (Fig. 3B; Tab. 2). The maximum constriction varied between 70% (baboon) and 81% (cynomolgus macaque). EC₅₀ values of marmosets (6.1 nM) and cynomolgus macaques (3.1 nM) were very similar to those of humans (2 nM; Table 2; Ref. 43). Only the baboons showed, however, a significantly higher EC₅₀ value of 64 nM.

In comparison to NHPs and humans (43), rodents (23, 32, 33, 59) and guinea pigs (43) showed always a maximum airway constriction of 100% in the case of bronchoconstriction (Fig. 4B). Furthermore, rodent PCLS were completely unresponsive to histamine and LTD₄. Serotonin, however, was a strong bronchoconstrictor in rodent and guinea pig airways (Fig. 4, A and B) in contrast to human and NHP airways.

DISCUSSION

Suitable animal models of lung diseases, which are predictive for clinical efficacy of biopharmaceuticals, are necessary to further improve current therapies (3). Due to their phylogenetic proximity to humans, NHPs are promising animal models for human lung diseases. NHPs are, however, highly developed animals exhibiting cost-intensive breeding and husbandry conditions, which make them very valuable. Despite increasing debate on animal experimentation, the most common areas of nonhuman primate research in the last decades were infection biology, neuroscience, and biochemistry/chemistry (10). Particularly with regard to ethical concerns, the development of experimental alternatives to in vivo studies of NHP is necessary. Like other in vitro/ex vivo methods, PCLS cannot completely replace in vivo models due to limited culturing time and the lack of a circulation and edema formation. As an organ model, however, PCLS have the potential to reduce animal numbers in preclinical studies, one lung or lung lobe gives sufficient material (>30 PCLS with centered airways) to study different substances in different concentrations. Thus the technique of PCLS reduces the numbers of required animals. In the present study, mechanisms of bronchoconstriction of four NHP species were compared in ex vivo experiments by using viable lung tissue sections of intermediate sized airways. We showed that all human-relevant bronchoconstrictors except LTD₄ elicited airway narrowing in all NHP PCLS, which suggests they

Table 2. Mediator-induced bronchoconstriction in NHPs in comparison with human data

	Methacholine	Histamine	Serotonin	LTD ₄	U46619	Endothelin-1
Baboon						
EC ₅₀ , nM	25	244	29	0.7	0.03	64
pD ₂	7.6 ± 0.2‡	6.6 ± 0.2†	7.5 ± 1.2	9.2 ± 0.5*	10 ± 0.6	7.2 ± 0.4‡
C _{max} , %	85 ± 13	90 ± 8*	29 ± 27	49 ± 34	91 ± 3	70 ± 32
Marmoset						
EC ₅₀ , nM	43	409	—	—	10.7	6.1
pD ₂	7.4 ± 0.2‡	6.4 ± 0.4†	N.C.	N.C.	8.0 ± 1.6	8.2 ± 0.3
C _{max} , %	74 ± 20	53 ± 22	13 ± 15	14 ± 10‡	69 ± 23	72 ± 21
Rhesus macaque						
EC ₅₀ , nM	195	894	544	—	N.D.	N.D.
pD ₂	6.7 ± 0.1	6.0 ± 0.2	6.3 ± 0.6	N.C.	N.D.	N.D.
C _{max} , %	88 ± 8	77 ± 12	22 ± 16	3 ± 4†	N.D.	N.D.
Cynomolgus macaque						
EC ₅₀ , nM	718	3208	—	0.09	0.4	3.1
pD ₂	6.1 ± 0.1‡	5.5 ± 0.2	N.C.	10 ± 0.5‡	9.4 ± 0.6	8.5 ± 0.3
C _{max} , %	81 ± 11	81 ± 11	2.0 ± 2.0	44 ± 20	65 ± 31	81 ± 13
Human						
EC ₅₀ , nM	234	2422	N.C.	6	1	2
pD ₂	6.6 ± 0.1	5.6 ± 0.2	N.C.	8.3 ± 0.1	8.8 ± 0.2	8.6 ± 0.1
C _{max} , %	75 ± 8	59 ± 12	3 ± 3	63 ± 11	75 ± 18	81 ± 9

Data are presented as means ± SD. Half-maximal effective concentrations (EC₅₀), potency [pD₂: −(logEC₅₀)], and maximal response (C_{max}) of airways in precision-cut lung slices from different nonhuman primates (NHPs) statistically tested against the human data from our previous publication (43). LTD₄, leukotriene D₄; N.C., not calculated; N.D., not determined. **P* < 0.05, †*P* < 0.01, and ‡*P* < 0.005, by one-way ANOVA, Dunnett's multiple comparison test vs. human.

are a suitable model for studying pharmacological responses in respiratory diseases.

Methacholine and histamine are routinely used as bronchoconstrictors in human clinical trials to measure nonspecific bronchial reactivity and to assess the efficacy of therapeutic agents (35). Methacholine, acting on muscarinic (M) receptors, causes bronchial smooth muscle contraction via M₃ receptors in most species, including guinea pigs, rodents, and humans (19, 23, 35). Histamine, acting on H₁ receptors, causes airway constriction in guinea pigs and humans (43) but is ineffective in most rodents (12, 23). In all NHP PCLS, methacholine and histamine induced a concentration-dependent increase in maximum constriction. This is in line with changes in lung function parameters in humans and anesthetized baboons, rhesus macaques, and cynomolgus macaques after histamine or methacholine challenge in vivo (13, 34, 36, 44, 46). Further, pulmonary function studies (13) in humans showed a strong correlation between the severity of airway responses to histamine and methacholine by producing a PC₂₀ with a coefficient of correlation (*r*²) of 0.85 and a slope of 1. This is also true for human PCLS with an *r*² of 0.99 (unpublished data). PCLS from baboons, rhesus macaques, and marmosets, but not from cynomolgus macaques, exhibit a linear correlation between methacholine and histamine treatment, with the coefficient of correlation varying between 0.92 and 0.99. Only marmoset monkeys, however, showed a slope of 1 like humans. Thus these data suggest a role of histaminic and muscarinic receptors in marmoset lungs comparable to that in humans.

The role of serotonin, a rodent mast cell product (4), in human asthma is doubtful. Serotonin causes no bronchoconstriction in healthy subjects in vivo (8) and in human PCLS (43), and only slight bronchospasm in asthmatics, with no significant changes in FEV₁ after serotonin challenge (51). Among NHPs, only the airways of baboons and rhesus macaques showed weak constriction after treatment with a high concentration of serotonin (Fig. 4). Thus serotonin plays only

a minor role in airway constriction in NHPs. In contrast, PCLS from guinea pigs and rodents showed almost complete constriction after treatment with 10 μM serotonin (Fig. 4A) (43, 58).

Lipid mediators such as cysteinyl (cys) leukotrienes, thromboxane, or prostaglandins play important roles in the pathogenesis of asthma (4, 22) and are involved in bronchoconstriction and allergic inflammation. The cys-leukotrienes LTC₄, LTD₄, and in part LTE₄ induce constriction of the airways by activating at least two receptors, cys-LT₁ and LT₂ (29). Selective LT₁ receptor antagonists, such as montelukast or zafirlukast, and LT synthesis inhibitors have shown clinical efficacy in the treatment of asthma (31, 47, 49). In human PCLS, LTD₄ showed a concentration-dependent induction of airway constriction (43). Cynomolgus macaque and baboon airways responded very sensitively to LTD₄, in contrast to marmoset and rhesus macaque airways, which were nonresponsive, similar to murine airways (Fig. 4) (23). Compared with guinea pigs (43), the cynomolgus macaque and baboon airways were more sensitive to LTD₄, although, unlike guinea pigs, they did not close completely. With regard to potency, the airway contraction in baboons was most comparable to humans. To our knowledge, the effect of LT on baboons and marmosets has not yet been investigated. However, our data are in line with previous data for cynomolgus and rhesus macaques. In vivo studies (30, 38) in cynomolgus macaques showed an increase in pulmonary resistance and a decrease in dynamic lung compliance after treatment with aerosolized LTD₄. Therefore, similar airway pharmacology between cynomolgus macaques and humans might be expected (29, 30). Furthermore, the LTD₄ receptor antagonist ICI 198615 reduced airway hyperresponsiveness in cynomolgus macaques (52) but not in rhesus macaques (40). LTD₄ challenge in healthy rhesus macaques (41) also revealed no changes in pulmonary function parameters (i.e., breathing frequency, pulmonary resistance, or dynamic compliance). Taken together, our findings suggest that

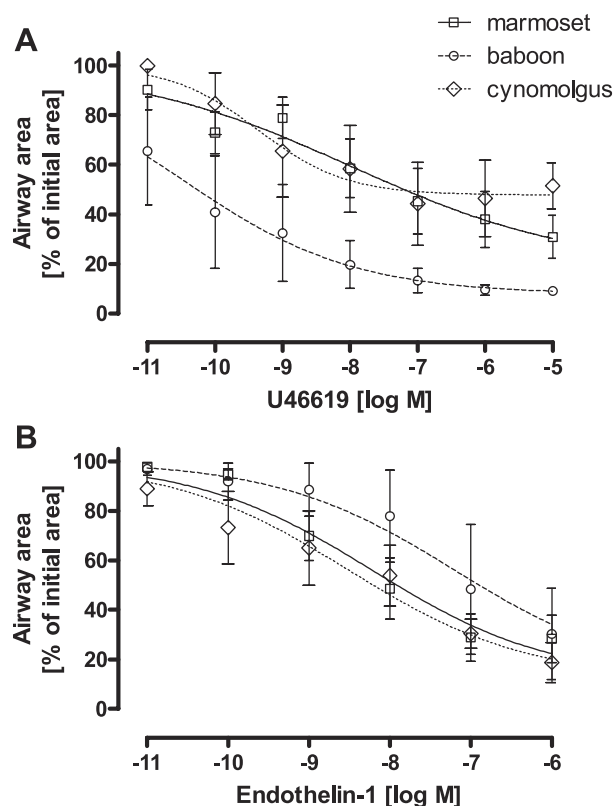


Fig. 3. Effects of U46619 and endothelin-1 on bronchoconstriction of baboon, marmoset, and cynomolgus macaque. Concentration-response curves for the following: A: U46619 [baboon ($\circ$): $n = 4$, $EC_{50} = 0.03$ nM; marmoset ($\square$): $n = 5$, $EC_{50} = 11$ nM; cynomolgus macaque ($\diamond$): $n = 5$, $EC_{50} = 0.4$ nM]; B: endothelin-1 ($\circ$): $n = 3$, $EC_{50} = 64$ nM; $\square$: $n = 4$, $EC_{50} = 6$ nM; $\diamond$: $n = 3$, $EC_{50} = 3$ nM). Data are expressed as means $\pm$ SE.

cys- LT_1 receptors are present in cynomolgus macaque and baboon airways but are absent in marmoset and rhesus macaque airways (Fig. 4). However, only baboons, one of the largest NHP species phylogenetically very close to humans, showed a bronchoconstriction comparable to that in humans after LTD_4 challenge.

The stable thromboxane-prostanoid receptor analog U46619 mimics the effects of thromboxane A_2 by activation of thromboxane-prostanoid receptors on airway smooth muscle cells, thereby causing airway constriction. So far, several thromboxane-prostanoid receptor antagonists have been developed and successfully tested for reducing or preventing human asthma, for example, seratroast, montelukast, and ramatroban (15). In humans, U46619 is a more potent bronchoconstrictor than methacholine, as assessed by lung function measurements in healthy and asthmatic subjects after inhalation of U46619 (27). In keeping with these findings, in all NHP PCLS U46619 was a more potent bronchoconstrictor than methacholine, according to their EC_{50} values that were 4 to 1,795 times higher for methacholine-induced than for U46619-induced bronchoconstriction (Fig. 4B). With the exception of marmosets, EC_{50} values in baboons, cynomolgus macaques, and human are ~ 1 nM with U46619, in contrast to methacholine which results in higher EC_{50} values varying between 25 and 718 nM. Previous ex vivo studies in rodent (23, 33) and guinea pig PCLS (43) reported EC_{50} values between 16 and 100 nM (Fig. 4). As shown in the present study, all tested NHPs might be useful

animals to investigate pharmaceuticals targeting thromboxane A_2 .

Endothelin-1, an endogenous peptide mediator, induced strong bronchoconstriction in NHP and human PCLS, with a maximum constriction of $\sim 80\%$ (Fig. 4A), whereas PCLS studies of guinea pig and rodent airways showed complete constriction of 100% after treatment with 1 μ M endothelin-1 (Fig. 4A) (23, 43). In addition, EC_{50} values after endothelin-1 treatment were similar in rodents, guinea pigs, humans, and NHPs, except baboons (Fig. 4B). To our knowledge, endothelin-1 has not yet been tested in any of the examined monkey species. In fact, Chalmers et al. (11) characterized endothelin-1

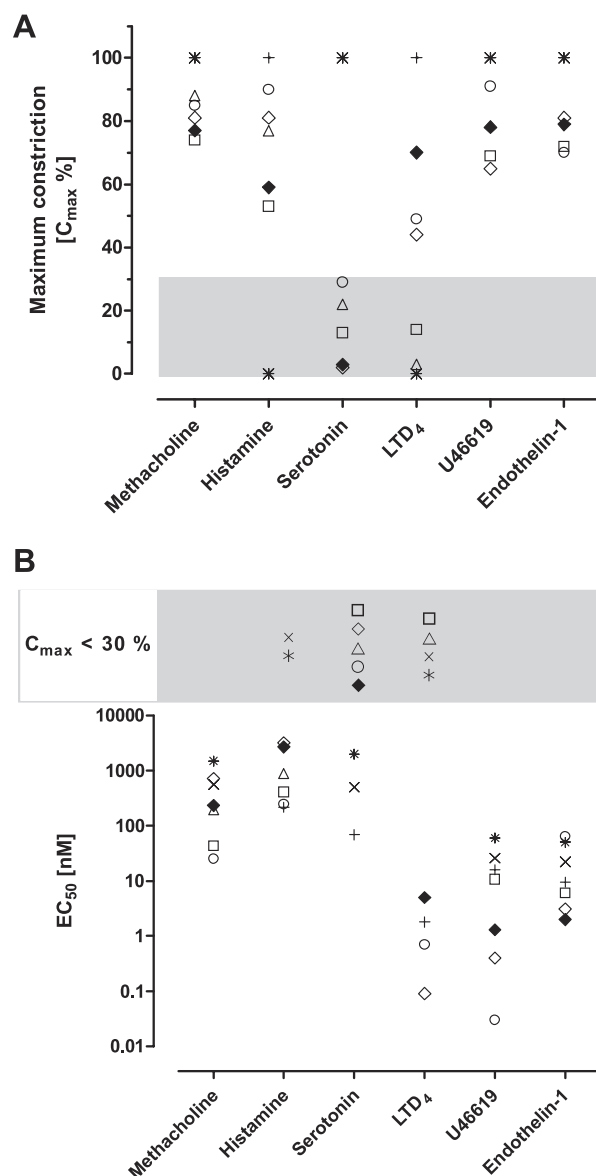


Fig. 4. Interspecies comparison of mediator-induced bronchoconstriction. Maximum constriction (C_{max} ; A) and EC_{50} values (B) for mediator-induced bronchoconstriction in marmoset ($\square$), baboon ($\circ$), cynomolgus macaque ($\diamond$), rhesus macaque ($\triangle$), human ($\blacklozenge$), rat ($\times$), mouse ($*$), and guinea pig ($+$) PCLS. Data of guinea pigs and humans have been taken from Ref. 43. Data from rats have been taken from Refs. 32, 33, 59. Data from mouse have been taken from Ref. 23. Symbols highlighted in grey represent the species showing a lower maximal constriction of 30%.

in a constant-volume body plethysmography in asthmatics, resulting in a 100-fold higher bronchoconstriction compared with methacholine. In PCLS, endothelin-1 also induced stronger bronchoconstriction than methacholine in all species except baboon (Fig. 4B). The distribution of endothelin type A and B receptors in NHPs is not known so far. In human lungs, the endothelin type A receptor is the predominant subtype on airway smooth muscles, but distribution and density of endothelin type A and B receptors can differ between species, for example, guinea pig and rat, and in location, for example, tracheal airway smooth muscle or lung alveoli (20). Regarding EC₅₀ values and potency, marmosets and cynomolgus macaques (Fig. 4) might be a suitable testing model for endothelin-1 antagonists because of their high sensitivity.

In the present study, peripheral airways showing residues of cartilage with intermediate size varying from 0.3 to 1.0 mm were used. However, the exact generations along the airway axis could not be determined. Differences between species in reactivity to various mediators may be due to anatomical differences or differences in the presence of various cells among the respiratory tract. For example, humans, cynomolgus macaques (42), and common marmosets (2) have cartilage in their airway walls up to the distal bronchioles and respiratory bronchioles, in contrast to rodents and guinea pigs, which show cartilage-free bronchioles (42). Another important fact is that in contrast to rodents, there are no inbred strains of NHP species. Moreover, the different strains were exposed to diverse environmental factors, like husbandry conditions (humidity or nutrition), social contact, and a different microbiological flora, factors that may influence response to bronchoconstrictors. Despite of these drawbacks, NHPs reflect the human situation more closely than rodents due to their close evolutionary relationship to humans making them indispensable for biomedical research, especially for the testing of biopharmaceuticals (10).

In conclusion, we were able to show that mechanisms of bronchoconstriction in NHP lungs share great similarities with human lungs. PCLS of all NHPs respond to mediators relevant to human airway disorders, such as methacholine, histamine, endothelin-1, and U46619, and are insensitive to the rodent mast cell product serotonin. Thus a more interesting species than rodents or guinea pigs for studying airway pharmacology are marmosets that share higher homologies to humans and are also easy to care and to handle due to their small size. Only PCLS from cynomolgus macaques and baboons, however, respond to leukotrienes like humans do, suggesting that among all compared species, these two NHPs resemble the human airway mechanisms best.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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