Minireview

Drug class effects on respiratory mechanics in animal models: access and applications

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Impact statement

Drugs such as anesthetics, muscle blockers, bronchoconstrictors, and bronchodilators are prevalent in respiratory mechanics studies. Each drug category plays a major role in these studies. Furthermore, the drug, dose, and injection via selection impact the mathematical modeling of the respiratory system, systemic conditions, and respiratory mechanics outcomes.

Abstract

Assessment of respiratory mechanics extends from basic research and animal modeling to clinical applications in humans. However, to employ the applications in human models, it is desirable and sometimes mandatory to study non-human animals first. To acquire further precise and controlled signals and parameters, the animals studied must be further distant from their spontaneous ventilation. The majority of respiratory mechanics studies use positive pressure ventilation to model the respiratory system. In this scenario, a few drug categories become relevant: anesthetics, muscle blockers, bronchoconstrictors, and bronchodilators. Hence, the main objective of this study is to briefly review and discuss each drug category, and the impact of a drug on the assessment of respiratory mechanics. Before and during the positive pressure ventilation, the experimental animal must be appropriately sedated and anesthetized. The sedation will lower the pain and distress of the studied animal and the plane of anesthesia will prevent the pain. With those drugs, a more controlled procedure is carried out; further, because many anesthetics depress the respiratory system activity, a minimum interference of the animal's respiration efforts are achieved. The latter phenomenon is related to muscle blockers, which aim to minimize respiratory artifacts that may interfere with forced oscillation techniques. Generally, the respiratory mechanics are studied under appropriate anesthesia and muscle blockage. The application of bronchoconstrictors is prevalent in respiratory mechanics studies. To verify the differences among studied groups, it is often necessary to challenge the respiratory system, for example, by pharmacologically inducing bronchoconstriction. However, the selected bronchoconstrictor, doses, and administration can affect the evaluation of respiratory mechanics. Although not prevalent, studies have applied bronchodilators to return (airway resistance) to the basal state after bronchoconstriction. The drug categories can influence the mathematical modeling of the respiratory system, systemic conditions, and respiratory mechanics outcomes.

Keywords: Pulmonary, engineering, lung, pharmacology, physiology, respiratory mechanics

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Introduction

The assessment of respiratory mechanics extends from basic research and animal modeling[1,2] to clinical applications in humans.[3,4] Related parameters, such as compliance/elastance and resistance, are recurrently assessed in intensive care units (ICUs). Recently, the assessment of respiratory mechanics has been in the spotlight due to Sars-CoV-2. However, to achieve its application in human models or in clinical practice, it is desirable and sometimes mandatory to study non-human animals.

However, a paradox described in the animal-model-related literature states that less invasive and less accurate methods (e.g., unrestricted plethysmography) and more precise and more invasive methods (e.g., positive pressure ventilation (PPV) with anesthesia and neuromuscular block) cannot be optimized simultaneously.[5] That is, by choosing greater precision in the evaluation, the condition of the animal utilized for modeling is more distant from its spontaneous ventilation.

Thus, to acquire more precise and controlled signals and parameters, the majority of studies use PPV with anesthesia to model the respiratory system during the respiratory mechanics evaluation.[6–8] In this scenario, a few drugs become relevant. These drugs can be categorized as anesthetics, muscle blockers, bronchoconstrictors, and bronchodilator. Although prevalent, several studies do not include the four categories in their study design. However, each drug category plays an important role in assessing respiratory mechanics. Hence, the main objective of this study is to review and discuss each drug category, and the impact of a drug on the assessment of respiratory mechanics.

Anesthetics

PPV is performed through commercial or experimental ventilators. Thereby, the animal must be orally intubated[9] or submitted to tracheostomy.[8] The animal utilized for the experiment must be appropriately sedated. The sedation will lower the pain and distress of the studied animal and the plane of anesthesia will prevent the pain, a more controlled procedure takes place, and there is minimum interference in the animal's respiration efforts because many anesthetics depress the respiratory system activity. The latter phenomenon is related to muscle blockers.

General anesthetics depress the central nervous system (CNS) adequately to allow surgery or invasive procedures. The anesthetics can be categorized as inhalational gases and parentally administered agents; the categorization is based on their structure and action mechanisms.[10–14] The existing literature presents certain options for anesthesia in invasive procedures for most studies on respiratory mechanics. Therefore, it is important to analyze different classes of anesthetics and their association with muscle relaxants, opioid analgesics, benzodiazepines, etc.

Halogenated

The halogenated inhalational class is among the most widely used surgical anesthetic classes.[15,16] The inhaled anesthetics molecular mechanism of action in the immobility and unconsciousness establishment is not completely understood. However, there is evidence that many receptors and channels are involved in these mechanisms, such as glycine receptors,[10] inhibition of N-methyl-D-aspartate (NMDA),[11] and inhibition of presynaptic sodium channels.[17] A few halogenated anesthetics have been described in the literature, such as enflurane, isoflurane, desflurane, and sevoflurane. Studies have shown that these anesthetics cause less liver damage when compared with halothane.[18] This is justified by the lower metabolism of the anesthetics through the CYP2E1 hepatic pathway.[18]

The study conducted by Llop-Guevara *et al.* uses inhalation and the assessment of respiratory mechanics through the constant phase model (CPM).[15] They computationally investigated the interaction between aeroallergens and immune system *in vivo*, where BALB/c mice were anesthetized with isoflurane (3% with 1 L/min of O_2). Certain studies have reported that inhaled anesthetics can interfere with the lung function. Fengxian *et al.*[16] demonstrated that in mice, isoflurane activates the transient receptor potential A1 (TRPA1), a non-selective, ligand-gated cation channel, which increases the rate of anesthesia onset. This activation caused strong nociceptive reflexes that reduced ventilation and airway compliance. These findings demonstrate that TRPA1 activation during isoflurane anesthesia impaired respiratory function and prolonged anesthesia induction latency. Other inhaled anesthetics seem to change respiratory function. Desflurane, which also activates TRPA1 channels, induces airway contraction and increases lung resistance in guinea pigs.[19] Hence, anesthetic selection may influence respiratory mechanics.

Ketamine

Ketamine is a general and dissociative anesthetic used in humans and animals. Additionally, it is used as an adjunct to local anesthetics in humans and non-human animals.[20] It is administered intramuscularly, intraperitoneally, or intravenously. An arylcyclohexylamine anesthetic, ketamine acts as a non-competitive inhibitor of NMDA receptors in the brain.[21,22] Apart from its action in NMDA receptors, ketamine also interacts with mu-, kappa-, and delta-opioid receptors, as well as monoaminergic, cholinergic, muscarinic, and nicotinic receptors.[12] Ketamine causes central sympathetic stimulation[23] and inhibition of catecholamine reuptake from adrenergic nerve-endings,[24] which results in higher blood pressure levels.

The ketamine application as an anesthetic agent is also combined with xylazine, which promotes muscle relaxation. This mixture is widely used for anesthesia in experiments that evaluate respiratory mechanics in small animals.[25–28] The required dosage depends on the animal species,[26,28] time required[29] for the procedure, and association with other agents.[30] Our laboratory has often used a combination of ketamine and xylazine to study respiratory mechanics in rats and mice. In these studies, we have evaluated airway resistance, tissue viscance, and tissue elastance using the CPM in animal models of Parkinson's disease, asthma, and epilepsy.[8,26–28] From the existing literature, intraperitoneal (i.p.) ketamine doses can range from 120 to 450 mg/kg.[31,32]

Barbiturates

These compounds induce sedative and hypnotic activities and act as non-selective CNS depressants.[33] Currently, they have been replaced by benzodiazepines,[33] which are more effective in promoting sedative and hypnotic actions. Barbiturates, particularly sodium pentobarbital, have been used for anesthesia in association with xylazine[34] or opioid agents[35] in studies pertaining to respiratory mechanics.

Xylazine

This is an agonist of alpha2 adrenergic receptors located in the central and peripheral nervous system,[36,37] which when stimulated, prevent the release of norepinephrine by inhibiting the influx of calcium ions into the cells.[38] The main effects of xylazine are sedation, analgesia, and muscle relaxation.[36] Xylazine can promote bradycardia by

increasing parasympathetic tone and decreasing central nervous system sympathetic activity[39,40] and hyperglycemia. Despite the bradycardia produced by the xylazine action on the nervous system, the hyperglycemia can produce tachycardia.[41] The hyperglycemia produced by xylazine is related to the alpha2 adrenergic receptors of pancreatic cells that inhibit insulin secretion.[42]

Ketamine causes central sympathetic stimulation[23] and inhibition of catecholamine reuptake from adrenergic nerve endings,[24] which results in higher blood pressure levels. However, the combination of ketamine and xylazine can partially mask these effects.[43] The administration routes in animals include intravenous, intramuscular, and subcutaneous administration. It is common to use an intraperitoneal route to induce anesthesia with ketamine in association with xylazine.[27,28,32] Like ketamine, the dose of xylazine depends on the animal species,[26,28] time required for the procedure,[29] and association with other agents such as anesthetics and opioid analgesics.[30]

Benzodiazepines

These have been widely used in veterinary medicine as an anti-epileptic agent and a pre-drug in inducing ketamine anesthesia.[44] Their effects are exerted via allosteric modulation of gamma-amino butyric acid (GABAA) receptor producing anxiolysis, amnesia, muscle relaxation, hypnosis, and anti-epileptic effects.[44] Benzodiazepines, such as diazepam, can be used to promote sedation in animals before inducing anesthesia.

Acepromazine

This is a phenothiazine derivative that promotes sedation via antagonism of dopamine D_2 receptor in the basal ganglia and limbic system.[45] It further antagonizes histaminic, muscarinic, serotonergic, and $alpha_1$ receptors resulting in an antihistaminic effect, which is a centrally and peripherally mediated anti-emetic effect in addition to the antiarrhythmic effect.[45] Using acepromazine 20–30 min before inducing anesthesia in animals may be recommended by an ethical committee as it can promote less stress in handling the animal. However, studies should be conducted to appropriately understand the acepromazine interference with respiratory mechanics in experiments involving cholinergic agonists with action on muscarinic receptors, such as methacholine.

Opioids

For improved animal welfare, the application of anesthetics has been associated with other drugs, such as opioid agents. Fentanyl is an opioid analgesic drug, which is a synthetic phenylpiperidine derivative with potent mu-opioid receptor activity. Certain fentanyl derivatives also act on delta- and kappa-opioid receptor activity.[46] It is highly lipophilic and easily absorbed by biological membranes with rapid penetration into the CNS.[47] It produces rapid and effective analgesia, and is 100 times more potent than morphine.[46]

Muscle blockers

Neuromuscular blockers (NMBAs) act on nicotinic cholinergic receptors at the neuromuscular junction to impair the transmission of nerve impulses, which result in muscle relaxation.[48] Currently, there are several drugs that belong to the NMBAs class; however, pancuronium, vecuronium, and rocuronium do not seem to interfere with lung function.[49] Routes of administration are generally intravenous and intraperitoneal; doses vary widely.[26–28]

NMBAs are common in studies involving the evaluation of respiratory mechanics. The purpose of this application is to minimize respiratory artifacts that may interfere with forced oscillation techniques (FOTs).[50] The muscle blockers directly influence the system's linear or nonlinear behavior. Hence, for linear models based on FOT, such as the CPM, the application of a muscle blocker is highly recommended.[5] For example, if the animal exhibits voluntary respiration efforts, the output signal will not present solely the ventilator's perturbation input frequencies in the spectrum. For a linear behavior, the output signals (the measured signal as the trachea pressure) can be shifted, attenuated, or amplified only by the system under study, but the frequencies must not change.[51] In case of an alteration of the frequencies in the output spectrum, the nonlinear behavior would increase and modeled parameters would become less reliable.

Bronchoconstrictors

The application of bronchoconstrictors is prevalent in respiratory mechanics studies. It is often necessary to challenge the respiratory system to verify differences among studied groups. For example, the PEEP values may be changed or bronchoconstriction may be pharmacologically induced. However, the selected bronchoconstrictor, its doses and administration can affect the evaluation of respiratory mechanics.

Acetylcholine

This transmitter is released from neurons, airway epithelial cells, and inflammatory cells[52,53] and binds to muscarinic receptors in the airways to trigger smooth muscle contraction and mucus secretion.[52] There are several muscarinic receptors identified thus far, which belong to the family of receptors coupled to the G protein.[54] However, only the M1, M2, and M3 receptors have been shown to play important roles in airway physiology and diseases, such as asthma and COPD.[54] The M3 receptors are the primary subtype of receptors for the contraction of bronchial smooth muscle, which are found in airway smooth muscle and submucosal glands.[52,54]

Methacholine

It is a cholinergic agonist that acts on muscarinic receptors, mainly subtype M3 coupled to G protein, promoting a cascade of events that lead to the recruitment of calcium with consequent contraction of smooth muscle.[55] The contraction of airway smooth muscle promotes a decrease in diameter with a consequent increase in airway resistance.[55] MCh

differs from ACh in terms of action time and selectivity, and it is degraded more slowly by acetylcholinesterase. In humans, MCh is often used in spirometry tests.[56,57] In animals, MCh is used to evaluate respiratory mechanics in models of pulmonary inflammation, such as asthma. In these tests, MCh can be administered via inhalation or intravenously; the doses used vary widely.[6,58]

MCh bolus

Between bolus and continuous infusion, i.e. the IV methods, the bolus protocol is the most prevalent method of administering MCh. The main characteristic of this protocol is the presence of a peak response. However, owing to the respiratory mechanics attributes, such as the perturbation and inter-perturbation time intervals, the real peak may not correspond to the maximal observed peak during the respiratory mechanics evaluation, as shown in Figure 1. This response was performed using data from a previous study.[32]

The bolus protocol is not the sole protocol to present a rapid increase and decrease as a response after MCh administration; the MCh i.h. also presents the effect. However, in mice,[6] the MCh via bolus leads to a more homogeneous bronchoconstriction when compared with i.h.

Additionally, in mice under severe bronchoconstriction, an increase in the harmonics distortion index was observed, which estimates the nonlinear behavior of the respiratory system near the observed peak value.[8] This information is relevant with respect to mathematical modeling because the CPM used in the later work is a linear model. Thus, in such cases, the CPM may be applied under non-ideal circumstances.[51] This nonlinear behavior can be influenced by the MCh dose,[8] and the doses used in bolus can be up to 3 mg/kg (Table 1). The nonlinearities increase with an increase in the dose.

Another intrinsic characteristic relative to the bolus protocol, and possibly the i.h. MCh, is the FOT. To model the respiratory parameters in an animal model, the respiratory system should be perturbed using an input signal. However, the amount of influence of the bronchoconstriction moment (during bronchoconstriction or relaxation), in which this perturbation is applied on mathematical modeling, is uncertain.

MCh infusion

The MCh continuous infusion protocol is less prevalent than the bolus protocol because it requires an infusion pump and additional attention to drug administration. An important characteristic of the infusion protocol is the "washout" required to begin the MCh infusion after the PBS or a saline solution. This may increase the total experiment time because all the previous residual content (in the cannula connected to the pump) is injected into the animal to introduce the actual MCh content.[32] The volume of the dose solution must be controlled to avoid considerable increase in the animal's volemia. The washout time depends on the cannula capacity and adjusted flow.

However, there are a few studies that apply the infusion technique.[63–66] The continuous infusion protocol selected in these studies may pertain to the mechanical ventilator/technique used (wavetube) and time length of the applied volume perturbation, which is up to 16 s each. The assessment of respiratory mechanics is generally conducted using the mean of three perturbations in the wavetube technique.

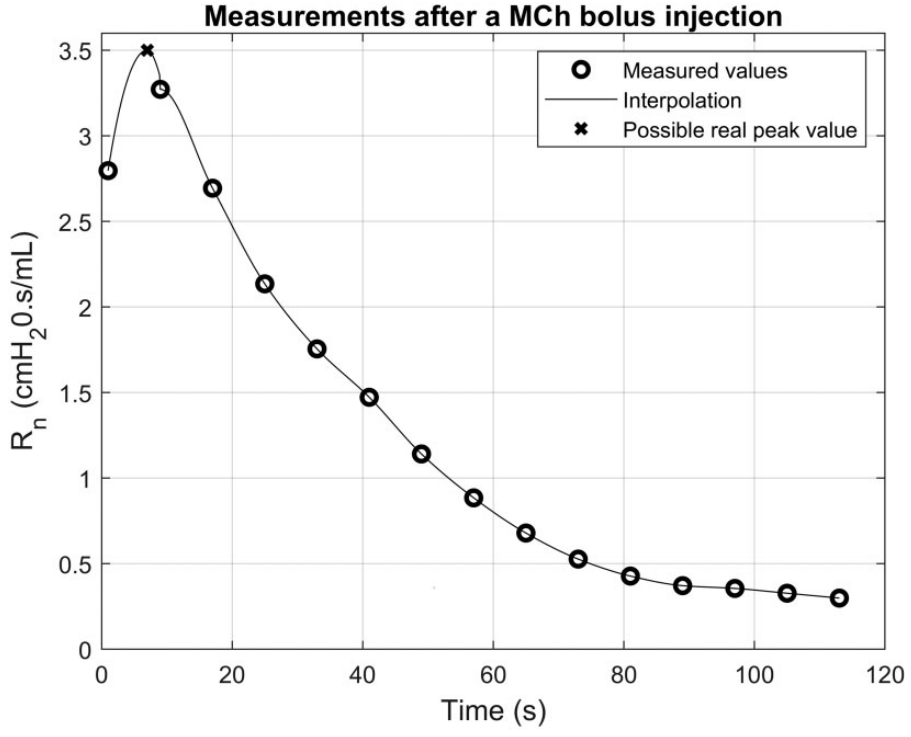

Figure 1. Bolus response after administrating 1 mg/kg of MCh. This depicts a possible peak loss.

Table 1. Different animal strains, MCh doses, and ventilators in the MCh bolus protocols.

Animal	Delivery of MCh	Ventilator	References
Mice BALB/c C57BL/6	i.v. in the lateral tail vein, doses of MCh (0.03, 0.1, 0.3, 1, and 3 mg/kg) at 4-min intervals.	flexiVent (SCIREQ)	6
Mice BALB/c C57BL/6	i.v. in the lateral tail vein, doses of MCh (0.03, 0.1, 0.3, 1, and 3 mg/kg) at 4-min intervals.	flexiVent (SCIREQ)	59
Mice SAMR1	i.v. in the jugular vein, doses of MCh (0.03, 0.1, 0.3, 1 mg/kg)	flexiVent (SCIREQ)	32
Mice BALB/c	i.v. in the jugular vein, doses of MCh (0.03, 0.1, 0.3, 1 mg/kg)	flexiVent (SCIREQ)	8
Mice Balb/cJ	i.v. in the jugular vein, half log doses of MCh from 10.0 to 316.2 μg/kg	flexiVent (SCIREQ)	60
Rats Brown-Norway	The chest was opened widely by midline sternotomy, and a venous line was established for the administration of saline and MCh. Bolus of MCh: 0.08 mg in 0.08 mL	flexiVent (SCIREQ)	61
Rats Wistar	i.v. in the jugular vein, doses of MCh (0.1–300 μg/kg)	Harvard 683 (Harvard Apparatus)	62
Rats Wistar	i.v. in the jugular vein, doses of MCh (0.3 and 300 μg/kg)	flexiVent (SCIREQ)	9

Because this perturbation requires longer time when compared with, for example, a 3-s perturbation, it is important that the respiratory system achieves a steady state during bronchoconstriction. This is only possible with a continuous infusion protocol.

The literature describes the achievement of stable serum MCh concentrations at dosages as high as $48\,\mu g \cdot kg^{-1} \cdot min^{-1}$, and the time required was 5 min.[65] The infusion protocol MCh doses can be up to 96 and $192\,\mu g \cdot kg^{-1} \cdot min^{-1}$.[32,64] The relevance of a steady state on a longer perturbation length (16 s) is because of the respiratory mechanics evaluation when the respiratory system may be transitioning from bronchoconstriction to bronchorelaxation within a 16-s window. Thus, the respiratory airway and parenchyma mechanics may change significantly with a longer perturbation time window, which is sufficient to adopt a continuous infusion protocol with longer perturbations.

Nevertheless, the protocol selection for MCh administration can impact respiratory mechanics. For example, a lower incidence of highly contracted airways was found in the continuous infusion group when compared with the aerosol group.[67] In addition, the continuous infusion protocol in rats[68] is responsible for the predominance of airway rather than parenchyma contraction when compared with the i.h. MCh administration.

Considering the bolus and continuous infusion protocols, our group recently published a paper comparing the protocols in mice during normal aging process.[32] We found that the bolus and continuous infusion protocols could differentiate the CPM parameters among different ages with four (30, 100, 300, and $1000\,\mu g \cdot kg^{-1}$ for bolus) and three (48, 96, $192\,\mu g \cdot kg^{-1} \cdot min^{-1}$ for continuous infusion) doses of MCh (Table 2). Additionally, the main parameter affected by the aging process was the elastance, and the observed differences were similar in the two protocols. Currently, we are investigating the influence of these protocols on the respiratory mechanics in an animal model of lung inflammation.

In addition, there was a lower degree of bronchoconstriction and number of ventilation defects (regions where ventilation is impaired or non-existent) [70,73] in the continuous infusion than for similar doses via bolus protocol.[70]

Although similar doses via bolus vs. infusion may be attainable, the equivalence between bolus and continuous infusion doses is yet to be understood. To completely model the dose equivalence, the drug absorption and metabolism should be identified.

MCh aerosol

The aerosol protocol consists of the delivery of MCh particles to the lungs through the breathing system of the ventilator for small animals connected to an aerosol generator device.

In contrast to the infusion protocol that induces a sustained contraction on the airways, i.h. generates heterogeneous responses in the pulmonary mechanical response.[6,68,69,74,75] Heterogeneity is a result of an uneven MCh delivery related to the device technology (aerosol quality), aerosol delivery system (ventilation profile and circuit), and deposition within the lungs (animal pulmonary anatomy and physiology).[7,76,77]

The widely employed devices use compressed air (jet nebulizers), vibrating piezoelectric crystals (ultrasonic), or piezoceramic mesh (vibrating mesh) to generate aerosol. Particle size is reported as the mass median aerodynamic diameter (MMAD) that influence aerosol deposition due to its inertial impact, gravitational sedimentation, and diffusion. Variable aerosol particle size has been reported between and within nebulizer models.[78,79]

Different protocols for aerosolized MCh in animals have been described in the literature. However, there was no detailed description of key points, such as ventilation pattern, nebulization setting, and particle size, which influence

Table 2. Different animal strains, MCh doses, and ventilators in the MCh infusion protocols.

Animal	Delivery of MCh	Ventilator	References
Mice BALB/c	i.v. in the jugular vein, doses of MCh (3–96 μg/min/kg) using a stoelting syringe pump (Wood Dale, IL, USA). MCh-induced constriction was reversed by i.p. injection of atropine sulfate (6 mg/kg).	flexiVent (SCIREQ)[a]	69
Rats Sprague-Dawley	i.v. in the jugular vein, doses of MCh (1, 2, 4, 8, or 16 μg/kg/min).	Harvard small-animal ventilator (not specified model)[b]	68
Mice SAMR1	i.v. in the jugular vein, doses of MCh (48, 96, 192 μg/kg/min), the concentration of the MCh solution was 320 lg/mL, infusion time 5 min, infusion pump (11 Plus, Harvard Apparatus, USA)	flexiVent (SCIREQ)[a]	32
Rats Brown Norway	MCh solution at a rate of 0.5 mL/kg/min for 6 min. MCh was administrated cumulatively in increasing concentrations (10^5 M to 10^1 M in 1/2 log increments)	model 682 (Harvard Apparatus)[c]	67
Mice BALB/c	MCh solution (80 mg/mL in 0.9% saline). The infusion experiments used flow rates of 0.3, 0.6, 0.9, and 1.2, 2.4, 3.6, and 4.8 mL/h	flexiVent (SCIREQ)[a]	70
Rats Sprague-Dawley	Doses of 2, 6, and 18 $\mu g/kg^{-1}\cdot min^{-1}$ (body weight)	model 683 (Harvard Apparatus)[b]	71
Mice BALB/c	Doubling doses (6–48 $\mu g/kg^{-1}\cdot min^{-1}$)	flexiVent (SCIREQ)[b]	65
Rats Han-Wistar Sprague-Dawley Harlan	MCh (3, 10 or 20 μg/kg per min) was administered as a 5 min intravenous infusion and NECA (0.01, 0.05 and 0.1 mg/kg) as an intravenous bolus	7025 Rodent Ventilator (UGO Basile)[c]	72
Mice BALB/c	Mch (48 $\mu g/kg^{-1}\cdot min^{-1}$) followed by i.p. atropine (6 mg. kg^{-1})	flexiVent (SCIREQ)[b]	66

[a]Respiratory mechanics signals acquired by flexiVent.
[b]Respiratory mechanics signals acquired by wavetube technique.
[c]Respiratory mechanics signals acquired by author's own data acquisition system.

the delivery and aerosol deposition. The insufficient information regarding the aerosol protocol affects the reproduction of experiments and comparison between them.

The assessment of respiratory mechanics was presented predominantly in relation to MCh increments, although factors such as ventilation pattern, delivered circuit, and particle size may affect the animal response. In Table 3, a few aerosol protocols are listed. A comparison among these is difficult owing to the differences in nebulizer models, ventilators for small animals, particle sizes, and ventilation profiles during nebulization. Moreover, neither of the studies validates the particle size.

Bronchodilator

In humans, bronchodilators are used in asthma treatment[84] or as a part of a spirometry evaluation.[85] However, in the assessment of respiratory mechanics in animal models, bronchodilators are generally used as a way to return (airway resistance) to the basal state after the bronchoconstrictor administration.[66,69] Not all respiratory mechanics studies use a bronchodilator, but it can provide relevant information in dose–response curve studies.

Beta-2 agonists

These act as ligands for adrenergic receptors and initiate a transmembrane signal cascade, which involves the heterotrimeric G protein, Gs, and effector, adenylyl cyclase. Adenylyl cyclase increases intracellular cAMP through ATP hydrolysis and activates cAMP-dependent protein kinase A (PKA). In airway smooth muscle, PKA phosphorylates Gq-coupled receptors cause a cascade of intracellular signals that promote reduction in intracellular Ca^{2+} or decrease in its sensitivity.[86] The change in Ca^{2+} is associated with the phosphorylation of the myosin light chain,[87] subsequently preventing the contraction of airway smooth muscle. Other actions have been proposed for beta-2 agonists. This class of drugs can promote anti-inflammatory effects within the smooth muscle of the airways by reducing the intercellular adhesion molecule-1, thus reducing the release of stimulating factors from granulocyte-macrophage colonies, stabilizing mast cell degranulation, and inhibiting multiple inflammatory factor pathways.[88]

Anticholinergic agents

These have been considered as the first choice of bronchodilator therapy for the treatment of COPD and asthma.[89] They antagonize muscarinic cholinergic receptors that are expressed in most cell types and mediate cell signaling for acetylcholine.[89] Cholinergic receptor antagonism can result in bronchodilation and promote anti-inflammatory actions by inhibiting the release of pro-inflammatory cytokines from the epithelial and immune cells.[90,91] A variety of

Table 3. Different animal strains, MCh doses, and ventilators in the MCh inhaled protocols.

Animal	Nebulizer	Delivery of MCh	Ventilation pattern during nebulization	Particle size	References
Mice (BALB/c, C57BL/6)	Aeroneb (Aerogen, Ireland)[a]	- Doses of MCh (1.67, 5, 25, 50, 100, and 200 mg/mL) diluted in PBS at 4-min intervals, nebulization time 10 s.	150 breaths/min, Vt:12 mL/kg, PEEP 3 cmH_2O	4–6 μm[b]	6
Rats (Sprague-Dawley)	Ultra-Neb 2000 (DeVillbiss Health Care, USA)[a]	- Doses of MCh (1, 2, 4, 8, or 16 mg/kg), bias flow, nebulization time 40 s for dose.	90 breaths/min, Vt 0.3 mL, PEEP 2.5 cmH_2O	–	68
Mice (BALB/c)	Ultra-Neb 2000 (DeVillbiss Health Care, USA)[a]	- Doses of MCh (0.001–30 mg/mL), bias flow, 2 min for dose.	450 breaths/min, Vt 8 mL/kg, PEEP 2 cmH_2O	–	69
Mice (BALB/c)	AeroSonic 5000 D (DeVillbiss Health Care, USA)[a]	- Doses of MCh in PBS at a concentration of 10 mg/mL, bias flow, nebulization time 20 breaths.	30 breaths/min, Vt 0.4 mL, PEEP 3 cmH_2O	–	80
Mice (A/J)	Aeroneb Lab Nebulizer System (Aerogen, Ireland)[a]	- Six increasing-concentration doses of MCh in pH-buffered saline, nebulization time 10 s, duty cycle 50 % (20 ms).	150 breaths/min, Vt 10 mL/kg, PEEP 3 cmH_2O, I:E 2:3	4–6 μm[b]	7
Mice (A/J)	Aeroneb Lab Nebulizer System (Aerogen, Ireland)[a]	- Six increasing-concentration doses of MCh in pH-buffered saline, nebulization time 10 s, duty cycle 50 % (20 ms).	50 breaths/min, Vt 30 mL/kg, PEEP 3 cmH_2O, I:E 2:3	4–6 μm[b]	7
Mice (A/J)	Aeroneb Lab Nebulizer System (Aerogen, Ireland)[a]	- Six increasing-concentration doses of MCh in pH-buffered saline, nebulization time 2.5 s, duty cycle 25 % (10 ms).	50 breaths/min, Vt 30 mL/kg, PEEP 3 cmH_2O, I:E 2:3	4–6 μm[b]	7
Mice (A/J)	Aeroneb Lab Nebulizer System (Aerogen, Ireland)[a]	- Six increasing-concentration doses of MCh in pH-buffered saline, nebulization time 10 s, duty cycle 50 % (20 ms).	150 breaths/min, Vt 10 mL/kg, PEEP 3 cmH_2O, I:E 2:3	2.5–4 μm[b]	7
Rats (Sprague-Dawley)	Ultra-Neb 100 (DeVillbiss Health Care, USA)[a]	- Dose of MCh (256 mg/mL), bias flow 0.2 L/min, nebulization time 3 min.	60 breaths/min, Vt 8 mL/kg, PEEP 3-4 cmH_2O	4.8 μm[b]	75
Mice (BALB/c)	PortaSonic 8500 C (DeVillbiss Health Care, USA)[a]	- Dose of MCh (12.5 mg/mL), nebulization time 40 s.	30 breaths/min, Vt 0.4 mL, PEEP 3 cmH_2O	–	81
Mice (BALB/c, C57BL/6, A/J)	Mystique (Airsep Corp., USA)[a]	- Dose of MCh (12.5 mg/mL), nebulization time 40 s.	30 breaths/min, Vt 0.4 mL, PEEP 3 cmH_2O	–	82
Mice (A/J, SWR/J)	Aeroneb Lab Nebulizer System (Aerogen, Ireland)[a]	- Doses of MCh (1, 3, 10, and 30 mg/mL), bias flow.	30 breaths/min, Vt 40 mL/kg, PEEP 0 cmH_2O	–	83

[a]Ultrasonic nebulizer.
[b]Values were not validated.
(–) No indication was found in the article.

anticholinergic drugs have been described for the treatment of COPD[92] and asthma.[93] In animals, the influence or absence of basal cholinergic airway smooth muscle tone on respiratory mechanics was determined by the injection of atropine.[66] In different species, the bronchodilator can be used to assess this basal cholinergic airway smooth muscle tone.

Conclusions

Anesthetics, muscle blockers, bronchoconstrictors, and broncodilators play an important role in the design and outcomes of the respiratory mechanics assessment. Each drug should be selected according to the disease, condition, or species being studied. Furthermore, the access via dose and drug injection time can directly influence the mathematical modeling of the respiratory system, systemic conditions, and respiratory mechanics outcomes.

AUTHORS' CONTRIBUTIONS

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DECLARATION OF CONFLICTING INTERESTS

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