Journal of Pharmacological and Toxicological Methods 61 (2010) 219–229

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Original article

Pharmacological validation of a telemetric model for the measurement of bronchoconstriction in conscious rats

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ARTICLE INFO

Article history:
Received 17 November 2009
Accepted 12 February 2010

Keywords:
Rat
Telemetry
Intrapleural pressure
Bronchoconstriction
Conscious
Head-out plethysmography
Respiratory assessment
Methods

ABSTRACT

Introduction: Telemetric measurement of intra-pleural pressure in conscious animals that are restrained in head-out plethysmography chambers enables determination of airway resistance. Originally proposed over 10 years ago, pharmacological validation of this technique is limited. Here airway resistance in conscious, instrumented rats was compared to measurement in anaesthetised rats via a fluid filled oesophageal catheter following administration of two different pharmacological agents. **Methods:** Male rats were implanted with telemetry devices and were trained to accept the restraint of head-out plethysmography chambers. A separate group of male rats were anaesthetised, placed in a body-enclosed plethysmography chamber and were prepared with a tracheal, oesphageal and jugular vein cannulae. Methacholine or NECA were given intravenously and changes in ventilation and airway resistance were measured. **Results:** The pressure signal obtained in the telemetered rats was found to be extremely variable. Variability was confounded by excessive struggling, particularly during the infusion periods. Misplacement of the pressure sensitive catheter tip and prior habituation to the chamber were not factors in signal variability. Consequently, no dose–response relationship to either pharmacological agent was established in this model. Dose-dependent increases in resistance to both methacholine and NECA were measured in anaesthetised rats using body-enclosed plethysmography. **Discussion:** Given the variability of the pressure signal within and between rats, the feasibility of a model in conscious rats for the measurement of airway resistance is questioned. Improved restraint methods or alternative models in conscious animals should therefore be explored. In the meantime, assessment of airway resistance is best confined to the anaesthetised rat.

1. Introduction

It has been more than 10 years since the description of a technique that allows assessment of airway tone in parallel with changes in respiratory rate and tidal volume (Murphy, Renninger, & Gossett, 1998). Such a model offers the opportunity to conduct a complete assessment of respiratory function in conscious rats. This is particularly favourable for the safety pharmacology discipline where it is mandatory to test the effects of new compounds on the rate and depth of breathing prior to first time in human administration. Although, it is widely recognised that drug-induced bronchoconstriction can be fatal and moreover is not limited to a particular pharmacological class of compound (Murphy, 2005), measurement of airway tone is not a requirement of the current regulatory guidelines (Anon, 2000).

The gold standard technique to determine pulmonary resistance in animals typically involves estimation of pleural pressure changes by direct measurement of oesophageal pressure, concurrently with measurement of airflow under anaesthesia (Likens & Mauderly, 1982; Palecek, 1969; Milic-Emili, Mead, Turner, & Glauser, 1964). Oesophageal pressure is usually measured by advancing an air-filled balloon (Milic-Emili et al., 1964) or water-filled tube (Palecek, 1969) into the lower third of the oesophagus. Due to the proximity of the oesophagus to the pleural space, changes in oesophageal pressure are regarded as a close surrogate for changes in pleural pressure (Benditt, 2005). Transpulmonary pressure, the driving force of breathing, can then be estimated using a differential pressure transducer to calculate the difference

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doi:10.1016/j.vascn.2010.02.008

between oesophageal pressure and pressure at the airway opening. Performing a tracheotomy using an endotracheal tube allows pulmonary airflow to be measured directly with a pneumotach. Whilst rats are able to breathe spontaneously under anaesthesia, it is usual practice to mechanically ventilate them and hence such models are not suitable for assessment of drug-induced changes in ventilation.

The model described by Murphy et al. (1998) uses a pressure sensitive catheter that resides below the serosal layer of the oesophagus to enable direct measurement of sub-pleural pressure. Rats are then restrained in head-out plethysmography chambers for measurement of ventilatory parameters. Integration of the pressure signal from the telemetry implant together with pulmonary airflow data from the plethysmography chamber thus allows direct calculation of airway resistance, a direct measure of bronchoconstriction (Renninger, 2006). Previously, measurement of pleural pressure has relied upon invasive techniques that require anaesthesia and can pierce the pleural space (Lai-Fook & Rodarte, 1991). Such invasive techniques limit animal reuse. Murphy et al. demonstrated reliable measurements of sub-pleural pressure up to 14 weeks post-surgery. Furthermore, the direct measurement of airway tone enables the investigator to depart from the use of the enhanced pause (PenH) calculated from whole body plethysmography (Hamelmann et al., 1997; Chong, Agrawal, Romero & Townley, 1998) or midexpiratory flow (EF_{50}) measured by head-out plethysmography (Glaab et al., 2001; Glaab et al., 2002), the reliability of which has been extensively questioned (Bates & Irvin, 2003; Lundblad, Irvin, Adler, & Bates, 2002; Bates et al., 2004; Lomask, 2006; Cohen et al., 2007; Lundblad et al., 2007; Ewart, Osei, & Valentin, 2009). Consequently, the model described by Murphy et al. offers significant advantages and challenged the dogma that measurement of lung function is a tradeoff between accuracy and invasiveness.

Head-out plethysmography involves restraining animals in a body chamber with their head protruding through a neck collar, thus creating an airtight seal around the thorax and abdomen. Tidal volume and respiratory rate are calculated directly from the changes in chamber pressure caused by the movement of the thorax during tidal breathing. However, the acute restraint causes a stress response that can be characterised by activation of the hypothalamic–pituitary–adrenal (HPA) axis and behaviours such as struggling, production of fecal boli and vocalisation (Grissom, Kerr, & Bhatnagar, 2008). These factors adversely affect respiratory recordings and because the frequency and extent of these stress-related behaviours vary between animals, data obtained can be highly variable. Habituation to the restraint over a number of training sessions has been shown to decrease stress related struggling (Narciso, Nadziejko, Chen, Gordon, & Nadziejko, 2003; Renninger, 2006; Harris et al., 2005; Grissom et al., 2008; Norton & Mason, 2008). However regimes involving short animal handling sessions prior to acute restraint or habituating animals less frequently, but with a greater interval between sessions, may also be sufficient to induce considerable reductions in corticosterone levels (Dal-Zotto, Marti, & Armario, 2002; Gadek-Michalska & Bugajski, 2003).

Despite the advantages of Murphy's model over existing techniques, its use does not appear to be widespread. Indeed, only two publications exist that show a limited pharmacological validation of this model (Renninger, 2006; Norton & Mason, 2008) while 55 manuscripts on whole body plethysmography in rats and 843 manuscripts on rat telemetry have been published over the same period. The aim of this investigation was to establish the model described by Murphy and colleagues and pharmacologically characterise it using the adenosine receptor agonist NECA (5′-(N-ethylcarboxamido) adenosine) and muscarinic receptor agonist methacholine. The airway resistance values generated in the conscious telemetered rats were compared to those obtained in anaesthetised rats using body enclosed plethysmography.

2. Methods

2.1. Animals

All of the scientific procedures conducted on the rats in this investigation were performed under the authority of a UK Home Office Project Licence with appropriate ethical approval. A total of 60 rats were used. Twenty eight male rats were surgically implanted with a telemetry device (12 Han-Wistar, Alderley Park Biological Services; 6 Han-Wistar, Harlan UK; 10 Sprague-Dawley, Harlan UK), 32 rats were used in the optimisation of the habituation protocol (16 male Han-Wistar, Harlan UK; 16 male Sprague-Dawley, Harlan UK) and a further 46 male rats underwent dosing in body enclosed plethysmography (23 Sprague-Dawley, Harlan UK; 23 Han-Wistar, Harlan UK). Rats weighed 224 g to 385 g on the first exposure to either type of plethysmography chamber. They were housed in groups of three or four and had free access to irradiated pelleted food and water, except when they were within the plethysmography chambers. The holding rooms were illuminated by artificial light on a 12 h light/dark cycle and temperature and relative humidity were maintained at 19 °C to 23 °C and 40% to 70%, respectively. On arrival, rats were allowed a minimum of 4 days to acclimatise prior to any procedures being performed.

2.2. Intrapleural pressure measurements

Changes in pleural pressure were measured by pressure sensitive tips of surgically implanted telemetry devices (Model TA11PA-C40, Data Sciences International). Signals from the telemetry device were detected by receiver pads (Model RPC-1, Data Sciences International) and were analysed by DSI® Art Analogue 1.0 software before being integrated with synchronous ventilatory data using the Edacq data acquisition system (Version 1.8.1., EMMS) for real time calculation of pulmonary resistance.

Rats were anaesthetised using isoflurane (2–4% isoflurane mixed in 100% oxygen, supplied at approximately 3 L/min) and the fur shaved from the sternum to the inguinal area. Rats were surgically prepared under strict aseptic conditions with analgesia (0.01 mg/kg buprenorphine) given as directed by a veterinary surgeon. The area was then disinfected and a central abdominal incision made through the skin and along the linea alba of the musculature. The lobes of the liver were carefully held back using damp cotton swabs and the oesophagus isolated approximately 2 cm below the junction with the diaphragm. A trocar (20 gauge, 1.1 × 30 mm, BD Insyte) was inserted between the serosal and muscularis layers and tunnelled cranially past the juncture with the diaphragm and into the thoracic cavity. The trocar was removed and the catheter of the telemetry transmitter was inserted into the channel previously made by the trocar. The pressure signal emitted by the transmitter was monitored to confirm correct placement of the device. Upon attaining a pressure signal of maximal strength (amplitude of at least 20 cm H_2O), the catheter of the transmitter was secured in place at the entry point with medical grade tissue adhesive (Vetbond, 3 M) and a cellulose patch (DSI). The body of the transmitter, situated in the peritoneal cavity, was then secured to the abdominal musculature and the surgical site was closed in layers using sutures.

To aid recovery, rats were housed singly with soft bedding and received sub-cutaneous analgesic (0.01 mg/kg buprenorphine) approximately 7 to 12 h post-surgery. Body weights were measured daily and once fully recovered, rats were returned to a group-housing environment (usually 12 days post-surgery). Pleural pressures reported by the telemetry devices were frequently checked during recovery to monitor changes in signal quality.

2.3. Head-out plethysmography habituation

Prior to compound dosing, rats were habituated to the head-out plethysmography chambers. Habituation aimed to reduce (a) the