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Original article

# Respiratory function in rats restrained for extended periods: Assessment of the effects of bethanecol

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**Abstract**

**Introduction:** The ICH guideline S7A recommends that the effects of drugs on the respiratory system are evaluated in laboratory mammals prior to administration in man. Previously, animals have been placed in plethysmography chambers for short durations. This study investigates the possibility of restraining animals in chambers for a longer duration to assess respiratory function over extended periods. **Methods:** Respiratory function in conscious rats was assessed using plethysmography chambers where the rat body was enclosed in a sealed chamber while the head was free. Thoracic movements were measured by pressure transducers linked to a Buxco amplifier system and respiratory parameters were captured and analyzed by the Notocord HEM data acquisition system. Each animal was subjected to 5 acclimatization sessions of escalating duration (1, 2, 4, 5, and 6 hours (h)) over 5 days prior to testing, with a baseline recording session conducted the day prior to dosing. Animals (8 males/group) were dosed subcutaneously with saline or bethanecol (3, 10, or 30 mg/kg) and placed in the chambers for 6 h of continuous recording. Additionally, a recording session was conducted at 24 h post-dose. **Results:** Subcutaneous administration of 30 mg/kg bethanecol decreased respiration rate by up to 33% during the first 1.5 h post-dose and increased tidal volume by up to 46% from 0.25 to 1.25 h post-dose when compared to vehicle group data. A decrease in minute volume of up to 33% was observed 0.25 h following administration of the 10 and 30 mg/kg doses. **Discussion:** These data show a respiratory depression caused by the cholinergic agonist bethanecol, an effect partially compensated for by an increase in tidal volume. This also demonstrates the ability to continuously restrain and record respiratory parameters in conscious rats for up to 6 h without any negative impact on the quality of the data.


*Keywords:* Bethanecol; Methods; Minute volume; Plethysmography; Rat; Respiration rate; Safety pharmacology; Tidal volume

## 1. Introduction

Assessment of respiratory function is currently a component of the core battery of safety pharmacology studies determined by the International Conference of Harmonization (ICH). The ICH guideline S7A recommends that effects of drugs on the respiratory system are evaluated in laboratory mammals prior to administration in man. Conscious rodents, restrained in plethysmography chambers, have been used for studying the effects of drugs on respiratory parameters without the contra-indicating administration of anaesthesia (Murphy, Joran, & Renninger, 1993). Head-out, body-enclosed plethysmography chambers allow an accurate measurement of respiratory rate, tidal volume, and minute volume by measuring pressure changes within the chamber caused as a consequence of thoracic

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movement of the animal during respiration. Additional parameters such as inspiratory/expiratory time, peak inspiratory/expiratory flow, and end inspiratory/expiratory pause may also be measured using head-out, body-enclosed plethysmography chambers, however these parameters were not assessed during this study.

The purpose of this investigation was to assess the potential to restrain rats in head-out plethysmography chambers for extended durations of continuous recording. It has been established that rats can be restrained in nose-only exposure tubes for durations of up to 6 hours (h) (Mauderley, 1986), whereas head-out plethysmography chambers have been extensively used to assess respiratory function in rodents for shorter durations of less than 6 h (Glaab et al., 2001; Murphy et al., 1993). However, it is currently unclear whether rats will tolerate periods of extensive restraint in head-out chambers, for durations up to 6 h, without any detrimental effects on respiratory parameters. The muscarinic cholinergic agonist, bethanecol, which is a known bronchoconstrictor, was used as a positive control in this study to examine the influence of a compound with known respiratory effects on respiratory parameters acquired during an extended restraint period. Other well characterized respiratory depressants such as morphine or baclofen, or a respiratory stimulant such as theophylline, are standardly used as positive control substances in short duration respiratory studies.

## 2. Methods

This study was conducted under the UK Home Office Licence Number PPL 60/2765. This study was also conducted in compliance with Good Laboratory Practice (GLP) as required by the United Kingdom GLP Regulations 1999 (S.I. 1999 No. 3106) which are in accordance with the Organisation of Economic Co-operation and Development (OECD) Principles of GLP (ENV/MC/CHEM (98) 17). It is a general recommendation in the ICH S7A guidelines that assessment of respiratory function in conscious rats as part of the safety pharmacology core battery should be conducted in compliance with GLP.

### 2.1. Animals

Male Sprague–Dawley rats were supplied and delivered by Harlan UK Ltd. (Bicester, Oxon, UK) for use in this study. Each dosing group was allocated 8 animals. The rats were weighed prior to testing, and the body weights recorded, on the same day as the administration of substances. The weight range of the rats was 272–344 g and their age at the time of dosing was estimated at approximately 7–8 weeks. All animals were housed in a holding room for 1 day prior to the start of the acclimatization phase. The animals were housed in groups of 4 in solid-floor plastic cages with sawdust bedding. The rats were fed an expanded rodent diet of RM1(E) SQC (Special Diets Services, Witham, UK) ad libitum and allowed free access to mains tap water. The holding room had a 12 h light–dark cycle (on 07:00, off 19:00), and was air-conditioned by a system designed to maintain an air temperature of 20±3 °C.

### 2.2. Acclimatization

The rats were acclimatized to the plethysmography chambers on 5 separate occasions over 5 consecutive days prior to the first recordings, for increasing periods of approximately 1, 2, 4, 5, and 6 h. The acclimatization sessions were performed under normal lighting conditions, as per the recording sessions. Respiratory parameters were not recorded during the acclimatization sessions. Any clinical signs observed during the acclimatization sessions were noted.

### 2.3. Data acquisition

For each recording session, the animals were restrained in body enclosed plethysmography chambers designed to isolate the head from body respiratory movements. The chambers were attached to flow transducers connected to high gain amplifier units, which in turn were connected to an acquisition PC, from which changes in the rats' thoracic volume were measured. All recording sessions were conducted under normal lighting conditions. Acquisition and analysis of the respiration rate and tidal volume data was monitored using the Notocord HEM version 3.4 data capture system. Animals were placed in chambers for recording on 3 occasions over 3 days. On the day prior to dosing, baseline parameters were measured for at least 1 h. On the second day of recording, the animals were dosed and then placed immediately into the plethysmography chambers. Recordings were made for at least 6 h. On the third day of recording, parameters were measured for at least 1 h at approximately 23–24 h post-dose. Electronic markers were placed in the data file to define the point at which the data for each parameter were to be analyzed. Any clinical signs observed during the recording sessions were noted.

### 2.4. Data analysis

Respiratory parameters collected from each animal included tidal volume, respiratory rate and consequently minute volume. For each parameter, fourteen 15 minute (min) averages were obtained. These averages were taken from the following time intervals: one from the last 15 min during the baseline collection, eight from the 15 min intervals from 0 to 2 h post-dose, four from the last 15 min of each hour from 2 to 6 h post-dose from the Day 2 collection, and one from the last 15 min from the Day 3 collection. All data were extracted onto a customized spreadsheet (Microsoft Excel 8.0) and mean values calcu-