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Role of gender in basic cough research

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ABSTRACT

Cough hypersensitivity affects mainly postmenopausal women. Pathogenesis remains unknown in their case; therefore, an optimal treatment is unavailable. Only male guinea pigs are used in basic cough research and exclusion of females leads to gender bias. Nowadays, the efforts of grant agencies aim to support projects which take gender into account and involve both sexes to reduce gender bias. The aim of our pilot study is to assess the suitability of female guinea pig model in cough research. Cough response to citric acid (0.4 M) was obtained in female and male guinea pigs (n = 12 each). Reproducibility of cough response was tested four times a week apart. The cough was detected from the airflow traces and cough sound analysis. Our initial results show that cough response of female guinea pigs is higher than in males. Variability of females is also higher than in males. Further investigations are necessary to validate this model in different conditions to achieve gender equality in cough studies.

1. Introduction

Coughing normally serves to stop potentially harmful substances from being inhaled and clearing excessive secretions from the airways. On the other hand, cough in chronic respiratory tract diseases is a bothersome problem, yet it may still represent an exaggerated reflex. There are also patients who cough excessively, although there is no known pathological process in their airways, with an exception of airway nerves hypersensitivity. Such a condition was aptly named cough hypersensitivity syndrome (Morice, 2013). Exact pathomechanisms of hypersensitivity of airways afferents and mechanisms of desensitization are a subject of intensive studies.

Experimental cough research utilizes male Dunkin-Hartley guinea pigs as most common experimental animal. However, cough hypersensitivity syndrome affects mostly peri- and postmenopausal females and its pathomechanisms are not yet clearly explained (Morice, 2013). Several causes are to be implied, but effect of sex hormones and their decrease is being favoured. Varechova et al. have confirmed that the changes in cough sensitivity between boys and girls occur in puberty (Varechova et al., 2008), which would indirectly support the theory of hormonal influence and our other work also documents that cough response of young fertile women depends on hormonal status (Kavalcikova-Bogdanova et al., 2015). Several studies have documented these gender differences in humans (Demoulin-Alexikova et al., 2016; Kastelik et al., 2002; Roorda et al., 1993).

These are the reasons why the absence of knowledge of cough response of female guinea pigs, as well as female guinea pig as an experimental model for cough research is important and may help to overcome the gap between basic research and situation observed in clinical studies. Nowadays, the efforts of grant agencies aim to support those research projects within basic research which take gender into account and experimental procedures involve male as well as female individuals. Such research conditions hold great potential in reducing "gender bias" in biomedical research (Beery and Zucker, 2011).

Our aim was to test cough response of female Dunkin-Hartley guinea pigs to standard tussigen – citric acid in suprathreshold concentration and to compare basic parameters of cough response to those obtained in male guinea pigs to determine whether female guinea pigs could eventually represent valid model for basic cough research.

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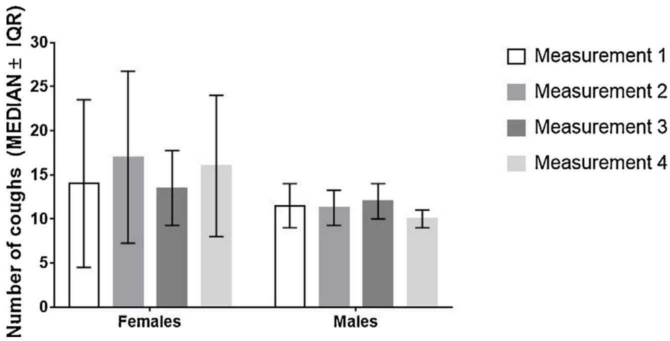

Fig. 1. Number of coughs obtained in repeated measurements of cough response in male and female groups of guinea pigs to 0.4 M citric acid for 10 min. Data are represented as median ± interquartile range.

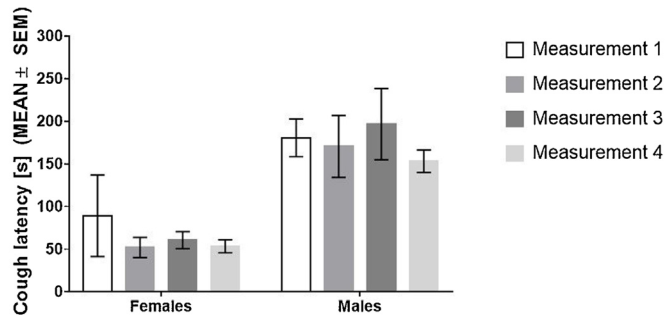

Fig. 2. Cough latency obtained in repeated measurements of cough response in male and female groups of guinea pigs to 0.4 M citric acid for 10 min. Data are represented as mean ± standard error of mean.

2. Material and methods

The study was conducted on male and female Dunkin-Hartley guinea pigs (males: n = 12, average weight 432 g, age 7 weeks at the time of first measurement; females: n = 12, average weight 371 g, age 7 weeks at the time of first measurement), obtained from an accredited breeding facility (Slovak Academy of Sciences, Dobrá Voda, Slovakia). The animals were housed in an approved animal holding facility maintained at a controlled room temperature of 21–22 °C, with humidity 50–60%, ventilation, a 12-h light–dark cycle, and with free access to water and a standard animal food.

Animal care was provided and the experiments were conducted in agreement with the Animal Welfare Guidelines of the Comenius University and the statutes and rules of the Slovak Republic (protocol no 3467/13-221).

Adaptation of animals to stress was performed in two phases. The animals were in contact with laboratory personnel daily prior to experimental measurements, during which they were exposed to laboratory conditions for approximately two hours twice. During this adaptation period, they were also placed inside a plethysmographic box and inhaled aerosol of buffered saline for 2 min to familiarize with the procedure of inhalation itself. Every day prior to challenge, they spent at least 30 min in laboratory to adapt to laboratory conditions and reduce the stress from transport from the animal housing facility.

2.1. Cough challenges in conscious guinea pigs

The conscious animals were individually placed in the plethysmograph (type 855, Hugo Sachs Electronic, March, Germany) which consisted of a head and a body chamber. The opening between the head and body chambers was equipped with a plastic collar lining around the animal's neck to prevent communication between chambers. An appropriate collar size was chosen for each animal to prevent neck compression. The head chamber was connected to a nebulizer (Pari Provokation Test I, Menzel, Germany, manufacturer's specification: output 5 l min^{-1}, particle mass median aerodynamic diameter 1.2 μm). A suction device adjusted to the same input flow (5 l min^{-1}) was connected to the head chamber to maintain constant airflow through the chamber during the aerosol administration. Airflow changes were measured using a pneumotachograph (Godart, Germany) with a Fleisch head connected to the head chamber. These data were recorded with the acquisition system ACQ Knowledge (Biopack, Santa Barbara, CA, USA). Respiratory sounds, including sounds during coughing and sneezing, were recorded with a microphone placed in the roof of the head chamber and connected to a preamplifier and MP3 recorder. The pneumotachographical and microphone output were simultaneously recorded for the off-line analysis.

Cough challenges were performed using an inhalation of 0.4 M citric acid for 10 min without any pre-treatment. Cough challenges were performed week apart.

Cough was defined as an expiratory airflow interrupting the basic respiratory pattern accompanied by a coughing sound. Coughs were analysed (using cough-related sounds and airflow) by two trained persons who were blind to the gender of animals. The most important task of the observers was to differentiate coughs from sneezes and artefacts by the software Sonic Visualizer. Their results were compared and (if no statistically significant differences occurred) averaged.

2.2. Statistical analysis

For the evaluation of the statistical significance of the data from the cough studies, non-parametric non-paired tests and multiple comparison tests were used as appropriate. The data for the final cough count are expressed as median ± interquartile range. The data for cough latency are expressed as average ± standard error of mean. For the statistical analysis, one-way ANOVA was used. $P < 0.05$ was considered statistically significant.

3. Results

Our initial results show that cough response of female guinea pigs is higher than of male guinea pigs. Variability of female group is also higher than that of male group. Number of coughs during repeated measurements of cough response did not significantly differ (14 ± 9.50 vs 17 ± 9.75 vs 13.5 ± 4.25 vs 16 ± 8.00) in between measurements in female group. Similar number of coughs was measured during repeated measurements in male group (11.50 ± 2.50 vs 11.25 ± 2.00 vs 12 ± 2.00 vs 10 ± 1.00) – again, without significant difference (Fig. 1). Cough latency did not differ significantly between measurements in female group (89 ± 48 s vs 52 ± 12 s vs 61 ± 10 s vs 54 ± 8s); however, these values were lower than cough latency in repeated measurements in male group (180 ± 22 s vs 170 ± 36 s vs 196 ± 40 s vs 153 ± 13s) (Fig. 2). These data indicate increased airway sensitivity, as cough occurs much sooner after tussigen exposure in females than in males.

The statistical analysis of difference of cough response between groups (male vs. female guinea pigs) was not performed at the time due to lack of data. Within general biomedical research, the conclusion that there is a statistically significant difference indicates only that the difference is unlikely to have occurred by chance. It does not mean that the difference is necessarily large, important, or significant in the common meaning of the word and it should not be the only part of the data analysis – biological relevance of the data should be considered as well (Lovell, 2013). However, the coefficients of variance were calculated for each measurement – both for number of coughs (ranging from 36.5% to 57.6% in female

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