Respiratory Physiology & Neurobiology 264 (2019) 8–11

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Short communication

Minute ventilation during hypoxia is augmented with capsaicin supplementation in aged mice

Candace N. Receno[a], Caitlin M. Cunningham[b], Keith C. DeRuisseau[c], Lara R. DeRuisseau[a,*]

[a] Department of Biological Sciences, Le Moyne College, 1419 Salt Springs Road, Syracuse, NY, 13214, USA
[b] Department of Mathematics, Statistics and Computer Science, Le Moyne College, 1419 Salt Springs Road, Syracuse, NY, 13214, USA
[c] Department of Exercise Science, Syracuse University, 201 Women's Building, Syracuse, NY 13244, USA

ARTICLE INFO

Keywords:
Unrestrained barometric plethysmography
VT/T_i
VE/VCO_2

ABSTRACT

Capsaicin is an agonist for transient receptor potential vanilloid 1 (TRPV1), and acute injection results in an increased frequency and tidal volume in young rats. It is unknown how capsaicin influences breathing in aged mice. We tested the hypothesis that capsaicin supplementation would elicit an augmented pattern of breathing in old mice compared to controls. Male 22-month old C57BL/6 J mice consumed a diet containing capsaicin (50 ppm) or lecithin control for one month. Breathing patterns were obtained prior to/following the dietary supplementation period using unrestrained barometric plethysmography. Frequency, tidal volume (VT), minute ventilation (VE), VE to expelled carbon dioxide ratio (VE/VCO_2) and VT divided by inspiratory time (VT/T_i) were analyzed at baseline and during a 15-minute hypoxic exposure (10% O_2). Capsaicin supplemented mice showed greater VE, VE/VCO_2 and TV/T_i during hypoxic exposure compared to controls, with no change at baseline. Overall, these findings suggest an acute augmented response to hypoxia following capsaicin administration in older mice.

1. Introduction

The respiratory system undergoes aging-related changes to compliance, overall structure, and muscle and lung function (see Sharma and Goodwin, 2006). Moreover, elderly men have a markedly reduced response to hypoxia compared to younger counterparts, suggesting a diminished hypoxic respiratory drive with age (Kronenberg and Drage, 1973). Since the aging population may be more susceptible to instances of hypoxia, the inability to elicit the appropriate protective breathing responses could be detrimental.

Transient receptor potential vanilloid subfamily member 1 (TRPV1) is a non-selective cation channel localized primarily in afferent sensory neurons, and is present throughout the respiratory system (see Tominaga and Tominaga, 2005). TRPV1 activation was shown to play a role in mediating respiratory inflammation in disease states, as it is expressed by sensory neurons within airways (see Gunthorpe and Szallasi, 2008). For this reason, the potential of TRPV1 antagonistic drug therapies continues to be explored in relation to cough, chronic obstructive pulmonary disease, and asthma (see Gunthorpe and Szallasi, 2008). However, TRPV1 agonists also show benefits to the ischemia-reperfusion model of lung injury. Capsaicin, an organic compound in spicy peppers, can act as a TRPV1 agonist. A study reported that rabbits pretreated with capsaicin prior to lung injury displayed improved gas exchange, as well as lower levels of inflammation and oxidative stress compared to controls (Wang et al., 2012). It was proposed that capsaicin activates TRPV1 channels within the sensory nerves of the respiratory system to release calcitonin gene-related peptide, resulting in decreases in inflammation and a subsequent protection from lung injury.

Capsaicin has historically been used as an aerosol irritant challenge in breathing studies (Bergren, 2001), but evidence also suggests that capsaicin administration via vascular injection results in altered ventilatory patterns. Through TRPV1 agonist actions, capsaicin changes the respiratory response to air when administered intravascularly shortly after birth and up to nine days following, in the form of augmented tidal volume and frequency (Kaczynska and Szereda-Przestaszewska, 2000; Wang and Xu, 2006). While these altered breathing patterns were documented following a single dose, the chronic effect of capsaicin supplementation on breathing is unknown. Measuring the control of breathing response could elucidate the potential of capsaicin to act as a therapeutic in aging and disease populations. The goal of this study was to document possible changes in the breathing pattern of aged mice

* Corresponding author at: Le Moyne College, 1419 Salt Springs Road, Syracuse, NY, 13214, USA.
E-mail addresses: recenocn@lemoyne.edu (C.N. Receno), cunninc@lemoyne.edu (C.M. Cunningham), kcderuis@syr.edu (K.C. DeRuisseau), deruislr@lemoyne.edu (L.R. DeRuisseau).

https://doi.org/10.1016/j.resp.2019.03.007
Received 4 February 2019; Received in revised form 4 March 2019; Accepted 18 March 2019
Available online 21 March 2019
1569-9048/

resulting from one month of capsaicin supplementation. We hypothesized that 22-month old C57BL/6 J mice exposed to dietary capsaicin for one month would exhibit an increased breathing response at rest and during hypoxic exposure compared to that of non-supplemented controls.

2. Methods

2.1. Animals

Senescent male C57BL/6 J mice were obtained from the National Institute of Aging, Aged Rodent Colony. Mice were housed under standard laboratory environmental conditions and a 12-hour light/dark cycle. Food and water were provided *ad libitum*. Mice were part of a study that also investigated the efficacy of capsaicin supplementation on aging muscle. Breathing data were also further analyzed in control animals for a manuscript describing baseline methods in aged mice. All use of animals was in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Le Moyne College Institutional Animal Care and Use Committee.

2.2. Diet

Mice were maintained on a standard chow diet (Teklad 2215 Diet, Envigo, Huntingdon, UK) during the first unrestrained barometric plethysmography test. Mice were subsequently divided randomly into two groups, provided either with the AIN-93 G diet (Harlan Laboratories, Madison, WI, USA) (n = 10; CON; TD.140388), or the AIN-93 G diet containing capsaicin (n = 8; CAP; 50 mg/kg food weight; TD.140389). Capsaicin was emulsified using lecithin and added to oil prior to integration into the pellet diet. CON diets also contained the same amount of lecithin and oil. Mice were provided the respective diets for one month, then unrestrained barometric plethysmography was performed again on the same mice.

2.3. Unrestrained barometric plethysmography

Mice were tested twice during the study using unrestrained barometric plethysmography to quantify breathing frequency (bpm), tidal volume (VT; mL/breath), minute ventilation (VE; mL/min), tidal volume to inspiratory time ratio (VT/T_i), and ratio of minute ventilation to expired carbon dioxide (VE/VCO_2). Unrestrained barometric plethysmography occurred prior to receiving the capsaicin or control diet at 22 months and again following one month of food supplementation at 23 months.

Testing conditions were similar to those previously described (Receno et al., 2018). Briefly, Mice were placed into a Buxco (Data Sciences International; DSI, St. Paul, MN) unrestrained barometric plethysmography chamber. A Validyne transducer (Validyne Engineering, Northridge, CA) was connected to the chamber for pressure measurements and was amplified using a signal conditioner. A barometric pressure, temperature and humidity probe was also fitted within the chamber for use in calculating tidal volume. Following baseline collection of frequency, VE, VT, VT/T_i and VE/VCO_2 in room air (20.93% O_2), mice were exposed to hypoxic gas (10% O_2, balanced nitrogen) for a total of 15 minute. Analysis of breathing data was performed using DSI Ponemah software and the Drorbaugh and Fenn equation. Data were analyzed during a 15-second quiet baseline (animals were awake with no active behaviors) and during 15-second intervals during hypoxic exposure at the five-minute, 10-minute, and 15-minute time points.

2.4. Statistical analysis

To confirm no differences between groups prior to administration of diet, a linear mixed model with a random effect for mouse was run with a between factor of diet group and a within factor of time (baseline, 5-minute, 10-minute and 15-minute hypoxic exposure), and with weight as a covariate. A Shapiro-Wilks test was employed to check for the normality of the data. Mice were only removed from the data set if they were considered to be a statistically significant outlier at baseline. The same linear mixed model was used to analyze the effects of one-month of capsaicin supplementation, with an added factor of pre/post capsaicin administration. If significant differences were found, post-hoc two-sample t-tests or a full linear mixed model (using only hypoxia time points) were run to further elucidate differences between groups. T-tests and a mixed model ANOVA were also employed to assess any differences in body weight within and between groups pre- and post-capsaicin administration. Changes within each group (CON or CAP) were also calculated by subtracting pre-diet from post-diet values for each mouse and variable of interest. Significance was set *a priori* at $p < 0.05$. Data are presented as mean ± SD.

3. Results

Initial breathing analysis performed between pre-CAP and pre-CON groups prior to the start of supplementation revealed no differences between the groups for any measures, including frequency, VT, VE, VE/VCO_2 and TV/T_i ($p > 0.05$; Table 1). Body weights of the pre-CAP and pre-CON groups were also not statistically different ($p > 0.05$, 34.5 ± 3.4 g and 34.1 ± 2.9 g, respectively).

Table 1. Frequency, tidal volume (VT), minute ventilation (VE), minute ventilation to expelled carbon dioxide ratio (VE/VCO_2) and tidal volume divided by inspiratory time (VT/T_i) in 22-month old mice prior to receiving capsaicin supplementation (Pre-CAP; n = 8) or lecithin control (Pre-CON; n = 10). Measures were collected during room air (20.93% O_2) and during the total 15-minute exposure to hypoxic gas (10% O_2) exposure. Values are displayed as mean ± SD. Mice were removed from the data set if they were found to be a statistically significant outlier. No significant differences between groups ($p > .05$).

Body weight was not altered as a result of the one month supplementation period, as there were no significant differences between CAP and CON ($p > 0.05$, 33.5 ± 3.3g and 34.9 ± 4.3g, respectively). Moreover, the change in weight pre- to post-supplementation was not different between groups ($p > 0.05$). After one month of capsaicin supplementation, no differences between CAP and CON groups for any of variables were observed during baseline breathing with room air. Importantly, differences did emerge during hypoxic gas exposure. VE

Table 1
Measures during air and hypoxic gas breathing in 22-month old mice prior to capsaicin supplementation.

	Frequency (bpm)	VT (mL/breath)	VE (mL/min)	VE/VCO_2 (ratio)	VT/T_I (mL/sec)
Room Air					
Pre-CAP	137 ± 24	0.35 ± 0.05	48.3 ± 9.8	110.5 ± 15.2	2.49 ± 0.73
Pre-CON	139 ± 9	0.38 ± 0.11	51.7 ± 12.9	109.9 ± 48.7	2.25 ± 0.86
Hypoxia					
Pre-CAP	254 ± 28	0.60 ± 0.12	147.8 ± 29.6	173.5 ± 35.0	5.87 ± 0.74
Pre-CON	270 ± 29	0.53 ± 0.10	141.3 ± 31.5	235.1 ± 61.9	5.55 ± 1.04

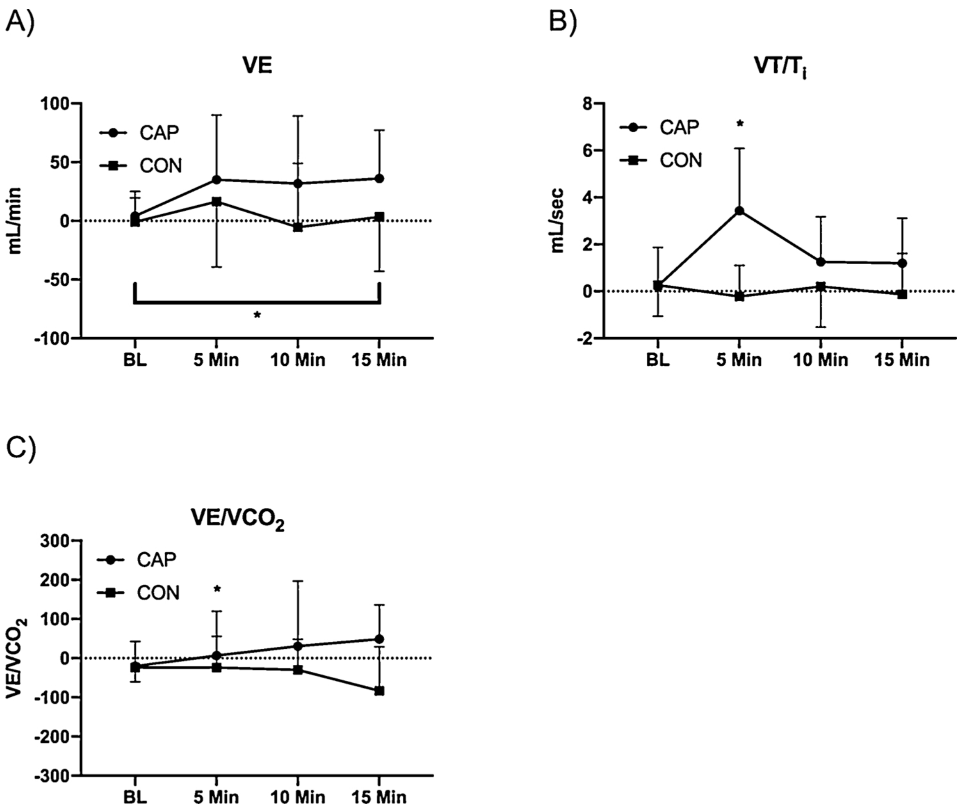

Fig. 1. Breathing measures in capsaicin supplemented (CAP; n = 8) and control (CON, n = 10) mice; post-pre group differences presented. A) Minute ventilation (VE) was significantly higher in CAP mice throughout 15 minute of hypoxic gas exposure post-supplementation compared to CON mice (*p = .015), B) Tidal volume/inspiratory time (VT/T_i) was greater at the five-minute hypoxia time point in CAP mice (*p = .007), and C) Ratio of VE to expelled carbon dioxide (VE/VCO_2) was higher at five minutes of hypoxic exposure in CAP animals (*p = .005). Data are presented as the mean difference (post-pre supplementation) ± SD for each measure prior to supplementation and after one month with or without capsaicin. BL = baseline, 5 Min = minute five of hypoxic exposure, 10 min = minute ten of hypoxic exposure, 15 min = minute 15 of hypoxic exposure. Mice were removed from the data set if found to be a statistically significant outlier at baseline. Mice (n = 3 CAP) were removed from VE/VCO_2 analysis.

was significantly different between the CAP and CON groups (p = .015; Fig. 1A), with the CAP mice showing a greater change in VE response during hypoxic gas exposure compared to CON animals. Changes in VT/T_i and VE/VCO_2 were also different between CAP and CON mice at the five-minute hypoxia time point (p = .007 & p = .005, respectively; Figure 1B&C), with a greater response during acute hypoxic exposure in the CAP group.

4. Discussion

Respiratory function has been shown to decline with age (Sharma and Goodwin, 2006). Since capsaicin has previously been observed to change breathing frequency and VE after a single injection in young animals, we were interested in the potential for chronic capsaicin supplementation to affect breathing in aged mice. We report that one month of dietary capsaicin supplementation resulted in a similar pattern of breathing during baseline between groups, but led to a greater VE, VT/T_i and VE/VCO_2 response with hypoxic gas exposure in the CAP mice.

Our findings at baseline suggest the increases in frequency and tidal volume previously observed after a single treatment (Kaczynska and Szereda-Przestaszewska, 2000; Wang and Xu, 2006) may be short lived and are not present with continued administration. Based on our data and these previous studies, another interpretation could be the response to capsaicin can differ amongst young and old animals. Importantly, changes in breathing were only uncovered with the use of hypoxic gas exposure. It is possible that the agonistic role of capsaicin on TRPV1 requires a challenge to the respiratory system to be revealed. A previous study in rats suggests that capsaicin blunts the response to hypoxia (Desanctis et al., 1991) when administered to pups as a means of desensitizing neurons. These rats were pretreated with capsaicin as pups (2 days postnatal), which resulted in a lower VE and longer inspiratory times compared to controls in response to hypoxic challenge. In contrast, our data in aged mice show that VE, VT/T_i and VE/VCO_2 are higher during hypoxic challenge. Hence, capsaicin may have negative consequences on protective breathing responses when administered during development, but provides an acute augmented hypoxic response during aging.

Overall, we report that capsaicin administration later in life does not result in any alterations to baseline breathing patterns, but results in an acute response to hypoxic gas exposure. Previous reports have shown capsaicin administration results in increases in frequency and VE, which could potentially be beneficial to the dysfunctional breathing profile found with aging. However, we report the impact of chronic supplementation is only viewed during short term challenges. This finding is still of importance during aging, as older individuals may be more susceptible to short hypoxic events throughout the day or night. Future work could investigate the effects of capsaicin supplementation in a model of chronic, intermittent hypoxia to elucidate other potential benefits.

Funding sources

This work was supported by the National Institutes of Health [R15 HD076379-S1].

Competing interests

The authors have no competing interests to declare.

Acknowledgements

The authors would like to thank Dr. Marisa Mickey, Angela Le, Sarah Ruby and Jacob Russell for their work to maintain the mouse colonies and for help with data analysis.

References

Bergren, D.R., 2001. Chronic tobacco smoke exposure increases airway sensitivity to capsaicin in awake guinea pigs. J. Appl. Physiol. 90, 695–704.
Desanctis, G.T., Green, F.H.Y., Remmers, J.E., 1991. Ventilatory responses to hypoxia and

hypercapnia in awake rats pretreated with capsaicin. J. Appl. Physiol. 70, 1168–1174.

Gunthorpe, M.J., Szallasi, A., 2008. Peripheral TRPV1 receptors as targets for drug development: new molecules and mechanisms. Curr. Pharm. Des. 14, 32–41.

Kaczynska, K., Szereda-Przestaszewska, M., 2000. Respiratory effects of capsaicin occur beyond the lung vagi in anaesthetized rats. Acta Neurobiologiae Experimentalis 60, 159–165.

Kronenberg, R.S., Drage, C.W., 1973. Attenuation of ventilatory and heart-rate responses to hypoxia and hypercapnia with aging in normal men. J. Clin. Invest. 52, 1812–1819.

Receno, C.N., Glausen, T.G., DeRuisseau, L.R., 2018. Saline as a vehicle control does not alter ventilation in male CD-1 mice. Physiol. Rep. 6.

Sharma, G., Goodwin, J., 2006. Effect of aging on respiratory system physiology and immunology. Clin. Interv. Aging 1, 253–260.

Tominaga, M., Tominaga, T., 2005. Structure and function of TRPV1. Pflugers Arch. Eur. J. Physiol. 451, 143–150.

Wang, R.R., Xu, F.D., 2006. Postnatal development of right atrial injection of capsaicin-induced apneic response in rats. J. Appl. Physiol. 101, 60–67.

Wang, M.H., Ji, P., Wang, R.R., Zhao, L.F., Xia, Z.Y., 2012. TRPV1 Agonist Capsaicin Attenuates Lung Ischemia-Reperfusion Injury in Rabbits. J. Surg. Res. 173, 153–160.