Respiratory Physiology & Neurobiology 270 (2019) 103267

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Short communication

The voltage-gated sodium channel $Na_V1.8$ blocker A-803467 inhibits cough in the guinea pig

M. Brozmanova[a,b], S. Svajdova[a,b], N. Pavelkova[a,c], Y. Muroi[d], B.J. Undem[d], M. Kollarik[c,*]

[a] Department of Pathophysiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia
[b] Biomedical Center Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia
[c] Department of Molecular Pharmacology & Physiology, Morsani College of Medicine, University of South Florida, Tampa, Florida, United States
[d] Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

ARTICLE INFO

Keywords:
Cough
Voltage-gated sodium channels
NaV1.8
Bronchopulmonary C-fibers
Vagus nerve

ABSTRACT

Cough in respiratory diseases is attributed to the activation of airway C-fibers by inflammation. Inflammatory mediators can act on multiple receptors expressed in airway C-fibers, nonetheless, the action potential initiation in C-fibers depends on a limited number of voltage-gated sodium channel (Na_V1) subtypes. We have recently demonstrated that $Na_V1.8$ substantially contributes to the action potential initiation in the airway C-fiber subtype implicated in cough. We therefore hypothesized that the $Na_V1.8$ blocker A-803467 inhibits cough. We evaluated the cough evoked by the inhalation of C-fiber activator capsaicin in awake guinea pigs. Compared to vehicle, intraperitoneal or inhaled A-803467 caused 30–50% inhibition of cough at the doses that did not alter respiratory rate. We conclude that the $Na_V1.8$ blocker A-803467 inhibits cough in a manner consistent with its action on the C-fiber nerve terminals in the airways. Targeting voltage-gated sodium channels mediating action potential initiation in airway C-fibers may offer a means of cough inhibition that is independent of the stimulus.

1. Introduction

The currently available antitussive drugs often have only a limited efficacy in inhibiting excessive cough in acute and chronic inflammatory respiratory diseases (Dicpinigaitis et al., 2014). Numerous inflammatory mediators act on multiple receptors on nerve terminals of airway afferent nerves leading to their depolarization and initiation of action potentials, which then propagate to CNS and trigger a cough reflex (Lin et al., 2017; Mazzone and Undem, 2016). Irrespective of the number and type of receptors engaged, the action potential initiation and propagation in afferent nerves depends on activation of a limited number of voltage-gated sodium channel subtypes (Na_V1s) (Bennett et al., 2019).

Extensive evidence indicates that cough caused by airway inflammation is mediated by the capsaicin-sensitive vagal afferent C-fibers which in the guinea pig are the C-fibers originating from vagal jugular ganglia (jugular C-fibers) (Mazzone and Undem, 2016). Of 9 known Na_V1 subunits ($Na_V1.1$ through $Na_V1.9$), only a handful are expressed in airway jugular C-fibers (Kollarik et al., 2018). We recently showed that $Na_V1.8$ substantially contributes to action potential initiation in the nerve terminals of airway jugular C-fibers (Kollarik et al., 2018). This finding predicts that the inhibition of $Na_V1.8$ in the airway nerve terminals will lead to the inhibition of cough. Because the airway nerve terminals can be targeted by drugs administered both systemically as well as topically to the airways, we hypothesized that both the systemic and inhaled $Na_V1.8$ inhibitor A-803467 (Jarvis et al., 2007) inhibits cough evoked by the C-fiber activator capsaicin in the guinea pig.

2. Methods

The protocol used to obtain tissues for patch clamp studies were approved by the Johns Hopkins Animal Care and Use Committee. Male Hartley guinea pigs (180–300 g, Hilltop Laboratory Animals, Inc., Scottsdale, PA, USA) were used. Standard whole cell patch clamp was performed to record sodium current as we described previously (Muroi et al., 2011). Briefly, nodose ganglia were dissociated and the recordings were performed within 24 h. The cells were superfused at the rate approximately 2 ml/min at room temperature with external solution for recording sodium current (in mM): 0.1 CdCl2, 1 CaCl, 10 Hepes, 1 MgCl2, 14 NaCl, 126 choline-Cl, 20 TEA-Cl and 3 KCl titrated to pH 7.3 with CsOH (320 mosmol/L, adjusted with D-glucose). Borosilicate

* Corresponding author at: Department of Molecular Pharmacology & Physiology, Morsani College of Medicine, University of South Florida, 12901 Bruce B Downs Blvd., MDC-4029, Tampa, FL, 33612, United States.
E-mail address: kollarik@health.usf.edu (M. Kollarik).

https://doi.org/10.1016/j.resp.2019.103267
Received 27 May 2019; Received in revised form 17 July 2019; Accepted 5 August 2019
Available online 06 August 2019
1569-9048/

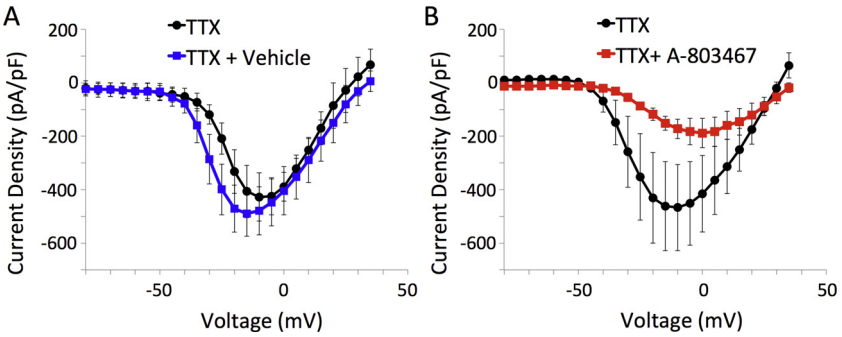

Fig. 1. The $Na_V1.8$ blocker A-803467 inhibits the tetrodotoxin (TTX)-resistant current in the guinea pig afferent neurons. Current density–voltage relationships of TTX (1μM) treated vagal nodose neurons in the presence of the vehicle (DMSO 0.3%, N = 3) and in the presence of A-803467 (30μM, N = 6).

patch electrodes with tip resistance of 1.0–3.4 MΩ were filled with pipette solution consisted of (in mM): 140 CsF, 1.1 EGTA, 10 Hepes, 0.1 CaCl2, 2 MgCl2 and 10 NaCl titrated with CsOH to pH 7.3 (310 mosmol/L, adjusted with D-glucose). Axopatch-800B amplifier with Axopatch and Axograph software (Molecular Devices, Union City, CA, USA) was used, with series resistance compensated at 70%. The neurons were incubated first with TTX (1μM) diluted in the external solution for 7 min and then with the combination of TTX (1μM) and A-803467 (30μM) or TTX (1μM) with vehicle (DMSO 0.3%) for 7 min. Sodium currents were measured after each incubation while holding the neurons at −80 mV, then prepulse potential of −120 mV (10 s) followed by a 50 ms depolarizing pulse from –100 to 35 mV in 5 mV increment. –P/6 subtraction was applied.

The cough reflex studies were approved by Ethical Committee of Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia. Male Dunkin Hartley guinea pigs were used (250–350 g Innovo, Isaszeg, Hungary). The animals were housed in air-conditioned room, temperature 22 ± 2 °C, relative humidity of 50 ± 5% with alternating 12 h light/dark cycle and provided with standard animal food and water ad libitum. The method used for capsaicin-induced cough was described previously (Brozmanova et al., 2012). Briefly, guinea pigs were individually placed into a double-chamber whole body plethysmograph (type 855, Hugo Sachs Electronik, March-Hugstetten, Germany). The head chamber was connected to the compressed air driven nebulizer (Metal Work, Pneumatic, Italy). A suction device adjusted to balance the nebulizer output was also connected to the head chamber to maintain constant airflow. Respiratory changes in the airflow were recorded using pneumotachograph Fleisch head connected to the head chamber and recorded and analyzed by Biopac system (Biopack Systems, Inc, Model MP100, Santa Barbara, CA, USA). Respiratory sounds including cough were recorded with a microphone placed in the head chamber connected to a preamplifier and MP3 recorder. Cough was detected during offline analysis as the expiratory airflow accompanied by the cough sound (detected by software Sonic Visualizer, Centre for Digital Music, Queen Mary, University of London, London, UK). Data are presented as mean ± SEM. Based on Saphiro-Wilk test (significance level 0.05) applied to control data, nonparametric and parametric tests were used for cough and respiratory rate, respectively, as indicated in the figure legend.

The animals were adapted to experimental conditions by performing inhalation of phosphate buffered saline (PBS) aerosol (5 min) twice on different days in the week prior to start of experiment. The animals in the first group received systemic intraperitoneal (i.p.) injection of A-803467 (50 mg/kg) or vehicle 30 min prior to inhalation challenge by aerosolized capsaicin (25μM) for 5 min. Because of unpaired design of this experiment, capsaicin-induced cough without any intervention was compared between the groups 7–10 days later and no significant difference was observed (data not shown). The second group of animals inhaled aerosol of A-803467 (1 mM) or vehicle for 10 min before the inhalation of capsaicin (25μM) containing A-803467 (1 mM) or vehicle for 5 min. This experiment had paired design in which two cough challenges were separated by 7–10 days. The animals received randomly A-803467 first or the vehicle first. The third group of animals underwent similar protocol except A-803467 was used in the concentration of 3 mM. The respiratory rate was determined by counting respiratory cycles over 1 min period. Because of continuous design of inhalation experiments the respiratory rate was determined in the last minute of the A-803467 inhalation. In the experiment with systemic i.p. A-803467 injection respiratory rate was determined during the first minute of capsaicin inhalation (in the first minute no cough was detected in 17 animals and only 1 cough was detected in 2 animals). For i.p. injections A-803467 was dissolved in the mixture of 5% dimethyl sulfoxide (DMSO)/95% polyethylene glycol PEG 400 to final concentration of 25 mg/ml (Jarvis et al., 2007). The drug solution or vehicle was injected i.p. in the dose of 2 ml/kg. For inhalation A-803467 was dissolved in DMSO to 100 mM and further diluted in PBS by rapid stirring, sonication and gentle warming to final concentration of 1 mM or 3 mM. Capsaicin was dissolved in ethanol to stock solution (0.1 M) and diluted to final concentration of 25μM in PBS or PBS containing vehicle or PBS containing A-803467 1 mM or 3 mM prior to experiment. DMSO, A-803467 and capsaicin, polyethylene glycol PEG 400 were purchased from Sigma-Aldrich (St. Louis, MO).

3. Results

We first evaluated the efficacy of A-803467 to inhibit the guinea pig $Na_V1.8$. We used vagal nodose neurons as the sodium current evoked in the presence of large concentration of tetrodotoxin (1μM) in these neurons (tetrodoxin-resistant sodium current, TTX-R) in the guinea pig was largely attributed to $Na_V1.8$ (Kwong et al., 2008). We found that A-803467 (30μM) indeed substantially inhibited, although did not eliminate, TTX-R current in the guinea pig (Fig. 1).

We next evaluated the effect of systemic administration of $Na_V1.8$ inhibitor A-803467 on capsaicin-induced cough in awake guinea pigs. In preliminary experiments A-803467 in the dose of 20 mg/kg intraperitoneally (i.p.) did not inhibit the capsaicin-induced cough (data not shown). We therefore selected the dose of 50 mg/kg i.p. consistent with ID_{50} values for in vivo studies in rat (Jarvis et al., 2007). In an unpaired study the inhalation of capsaicin (25μM, 5 min) caused substantially lower number of coughs in the group treated with i.p. A-803467 compared to that treated with i.p. vehicle (Fig. 2A, upper chart). In contrast, i.p. A-803467 did not reduce respiratory rate (Fig. 2A, lower chart, quantified at the beginning of capsaicin inhalation). Thus, the systemic administration of $Na_V1.8$ inhibitor A-803467 inhibited capsaicin-induced cough.

We then evaluated the effect of local administration (by inhalation) of $Na_V1.8$ inhibitor A-803467 on capsaicin-induced cough. A-803467 (1 mM) or vehicle aerosol was administered via inhalation for 10 min prior to and then during 5 min of capsaicin inhalation challenge (capsaicin was diluted in the A-803467 solution). This study had paired

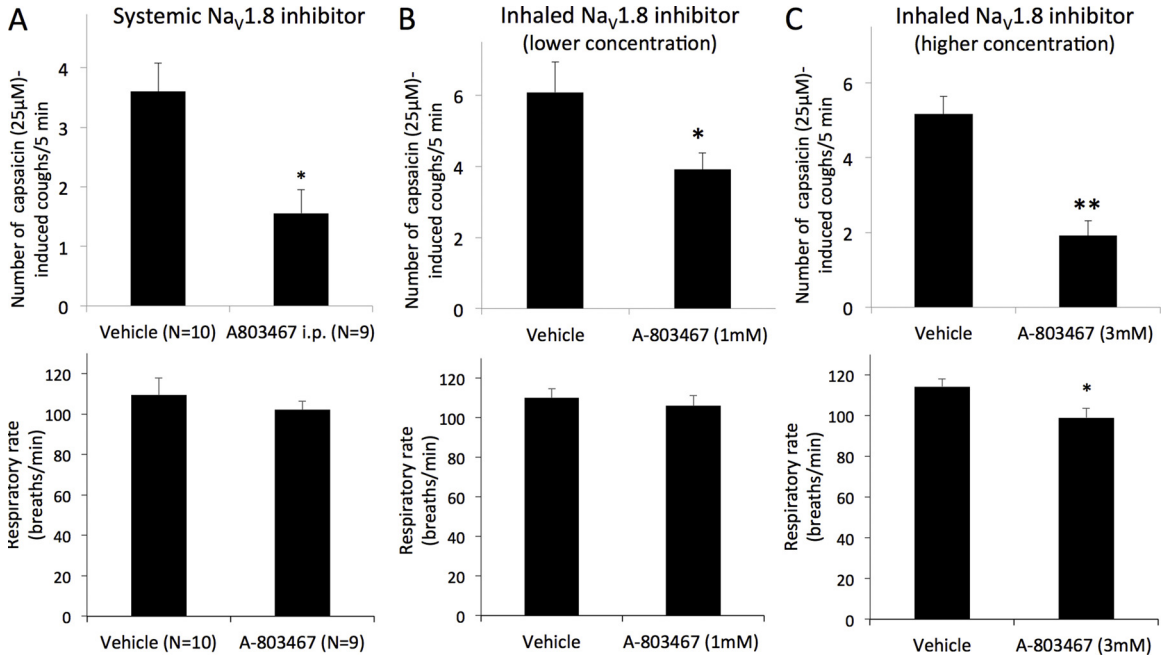

Fig. 2. Inhaled $Na_V1.8$ blocker A-803467 inhibits capsaicin-induced cough in awake guinea pigs. (A) Systemic intraperitoneal (i.p.) administration of A-803467 (50 mg/kg) substantially inhibited cough (upper chart, *P < 0.05, Mann-Whitney U Test) and had no appreciable effect on respiratory rate (lower chart, P = 0.5, unpaired T-test). (B) Inhaled A-803467 (1 mM) also inhibited cough (upper chart, *P < 0.05, Wilcoxon signed-rank test, N = 12) without affecting respiratory rate (lower chart, P = 0.6, paired T-test, N = 12). (C) At higher concentration A-803467 (3 mM) caused further inhibition of cough (upper chart, **P < 0.05, Wilcoxon signed-rank test, N = 12), but also inhibited respiratory rate (lower chart, *P < 0.05, paired T-test, N = 12).

design consisting of two capsaicin challenges separated by 7–10 days in which animals randomly received A-803467 or vehicle first. Inhaled A-803467 (1 mM) inhibited capsaicin (25μM, 5 min)-induced cough by ≈35% (Fig. 2B, upper chart) without an appreciable effect on respiratory rate (Fig. 2B, lower chart, determined at the end of A-803467 inhalation prior to capsaicin challenge). In a separate group of animals higher dose of inhaled A-803467 (3 mM) caused larger inhibition (by ≈60%) of capsaicin-induced cough (Fig. 2C, upper chart). Nonetheless, at this concentration of A-803467, a small but significant inhibition of respiratory rate by ≈15% was also observed (Fig. 2C, lower chart).

4. Discussion

We found that the $Na_V1.8$ inhibitor A-803467 reduced capsaicin-induced cough in the doses that did not affect respiratory rate. This could be achieved by either systemic or local (inhalation) administration of the drug. Our results are consistent with the hypothesis that $Na_V1.8$ in the nerve terminals of cough-initiating airway jugular C-fibers contributes to their activation.

There are at least two explanations why A-803467 inhibited, but did not abolish the cough response. Firstly, when drug solutions are applied as aerosols in our guinea pig cough model, the concentrations approximately 100-fold higher than those effective in isolated tissues are required because of the effects of distribution and dilution (discussed in detail in (Brozmanova et al., 2012)). Even at a relatively large concentration, A-803467 did not entirely inhibit the TTX-resistant (presumed $Na_V1.8$) current in guinea pig neurons in our patch clamp studies (Fig. 1). Secondly, based on our neurophysiological studies we expected that $Na_V1.8$ blockade may lead to only a partial reduction in action potential discharge, with more complete blockade requiring that both $Na_V1.8$ and TTX-sensitive Na_Vs (mainly $Na_V1.7$) are inhibited (Kollarik et al., 2018). Therefore, even if all $Na_V1.8$ channels were blocked it is unlikely that the capsaicin-evoked cough would be eliminated.

However, the expression and function of $Na_V1.8$ is upregulated in primary afferent neurons by inflammation (Bennett et al., 2019). In the somatosensory system, blocking $Na_V1.8$ has often limited effects on pain thresholds in healthy tissues, but inhibits the hyperalgesia associated with inflammatory pain (Bennett et al., 2019). Likewise, one might speculate that $Na_V1.8$ blockade may be more effective in normalizing the hyper-tussive state that often accompanies airway disease than in inhibiting cough in healthy individuals.

The systemic A-803467 can act on any part of a C-fiber (e.g. terminal, axon, cell body), however, the action of inhaled A-803467 is most probably limited to the C-fiber terminals in the airways. Therefore, the inhibition of cough by inhaled A-803467 is consistent with the inhibition of nerve terminals and in agreement with neurophysiological studies implicating $Na_V1.8$ in activation of nerve terminals. This leaves open the neurophysiologically justified possibility of topical (inhaled) administration of $Na_V1.8$ blockers in case preferred systemic administration leads to unacceptable inhibition of acute protective pain or nociceptive reflexes.

The numbers of coughs evoked by capsaicin in inhaled vehicle control groups (Fig. 2B-C) were higher than that evoked in the i.p. vehicle control group (Fig. 2A) probably because the vehicle (DMSO) was also present in the inhaled capsaicin aerosol in the former groups. Indeed, inhaled vehicle alone evoked on average 1 cough/5 min. The inhalation of A-803467 was immediately followed by the capsaicin cough challenge therefore the effect of inhaled A-803467 on respiratory rate was determined during the last minute of A-803467 inhalation. At the concentration of 1 mM inhaled A-803467 had no effect on respiratory rate. However, at the concentration of 3 mM inhaled A-803467 reduced respiratory rate by ≈15%. A likely explanation of this effect is the inhibition of vagal pulmonary mechanosensitive A-fibers (stretch receptors) which regulate breathing. Because of their vagal nodose origin and similarities to somatic mechanosensory A-fibers it is likely that these A-fibers depend on TTX-sensitive NaV1s and not on NaV1.8. Thus, the inhibition of respiratory rate at 3 mM of inhaled A-803467 could be caused by inhibition of other NaV1s by A-803467 (Stone et al., 2013). That inhaled A-803467 at lower concentration of 1 mM and the systemic A-803467 at the dose of 50 mg/kg did not inhibit respiratory rate is consistent with the lack of inhibition of pulmonary afferent fibers regulating breathing and selective (preferential)

inhibition of $Na_V1.8$. Nonetheless, the analysis of the effects of A-803467 and other NaV1.8 blockers on vagal bronchopulmonary fibers and other respiratory parameters is required to thoroughly address this issue.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Acknowledgement

This study was supported by the Department of Education of Slovakia [Grant VEGA No. 1/0020/19 and Grant Biomed Martin ITMS: 26220220187]. B. J. U is supported by NIHHL122228. M.K. is supported by NIHSPARC DK116311. The funding sources had no role in study design; in the collection, analysis and interpretation of data, in the writing of the report; and in the decision to submit the article for publication.

References

Bennett, D.L., Clark, A.J., Huang, J., Waxman, S.G., Dib-Hajj, S.D., 2019. The role of voltage-gated sodium channels in pain signaling. Physiol. Rev. 99, 1079–1151.

Brozmanova, M., Mazurova, L., Ru, F., Tatar, M., Kollarik, M., 2012. Comparison of TRPA1-versus TRPV1-mediated cough in guinea pigs. Eur. J. Pharmacol. 689, 211–218.

Dicpinigaitis, P.V., Morice, A.H., Birring, S.S., McGarvey, L., Smith, J.A., Canning, B.J., Page, C.P., 2014. Antitussive drugs–past, present, and future. Pharmacol. Rev. 66, 468–512.

Jarvis, M.F., Honore, P., Shieh, C.C., Chapman, M., Joshi, S., Zhang, X.F., Kort, M., Carroll, W., Marron, B., Atkinson, R., Thomas, J., Liu, D., Krambis, M., Liu, Y., McGaraughty, S., Chu, K., Roeloffs, R., Zhong, C., Mikusa, J.P., Hernandez, G., Gauvin, D., Wade, C., Zhu, C., Pai, M., Scanio, M., Shi, L., Drizin, I., Gregg, R., Matulenko, M., Hakeem, A., Gross, M., Johnson, M., Marsh, K., Wagoner, P.K., Sullivan, J.P., Faltynek, C.R., Krafte, D.S., 2007. A-803467, a potent and selective Nav1.8 sodium channel blocker, attenuates neuropathic and inflammatory pain in the rat. Proc Natl Acad Sci U S A 104, 8520–8525.

Kollarik, M., Sun, H., Herbstsomer, R.A., Ru, F., Kocmalova, M., Meeker, S.N., Undem, B.J., 2018. Different role of TTX-sensitive voltage-gated sodium channel (NaV 1) subtypes in action potential initiation and conduction in vagal airway nociceptors. J. Physiol. (Paris) 596, 1419–1432.

Kwong, K., Carr, M.J., Gibbard, A., Savage, T.J., Singh, K., Jing, J., Meeker, S., Undem, B.J., 2008. Voltage-gated sodium channels in nociceptive versus non-nociceptive nodose vagal sensory neurons innervating guinea pig lungs. J. Physiol. (Paris) 586, 1321–1336.

Lin, R.L., Gu, Q., Khosravi, M., Lee, L.Y., 2017. Sustained sensitizing effects of tumor necrosis factor alpha on sensory nerves in lung and airways. Pulm. Pharmacol. Ther. 47, 29–37.

Mazzone, S.B., Undem, B.J., 2016. Vagal afferent innervation of the airways in health and disease. Physiol. Rev. 96, 975–1024.

Muroi, Y., Ru, F., Kollarik, M., Canning, B.J., Hughes, S.A., Walsh, S., Sigg, M., Carr, M.J., Undem, B.J., 2011. Selective silencing of Na(V)1.7 decreases excitability and conduction in vagal sensory neurons. J. Physiol. 589, 5663–5676.

Stone, A.J., Kim, J.S., Yamauchi, K., Ruiz-Velasco, V., Kaufman, M.P., 2013. Attenuation of autonomic reflexes by A803467 may not be solely caused by blockade of NaV 1.8 channels. Neurosci. Lett. 543, 177–182.