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Abstract

Anandamide, an unstable arachidonate derivative, is originally identified as an endogenous ligand of cannabinoid receptor (CB) and, recently, is suggested to act as an endogenous ligand of vanilloid type 1 receptor (VR1 receptors). This study was carried out in anesthetized Sprague-Dawley rats to determine whether the pulmonary chemoreflex was altered by anandamide, Phenyl biguanide (PBG, 2-6 £g/kg i.v.), a selective stimulant of C fibers, injected at a dose just above the stimulation threshold elicited a mild respiratory and cardiovascular depression. In sharp contrast, during a constant infusion of anandamide (0.5 mg/kg/min i.v.), the same dose of PBG triggered a long apnea, accompanied by intense bradycardia and

hypotension. Similarly, the pulmonary chemoreflex response elicited by a bolus injection of adenosine and lactic acid was also greatly augmented by anandamide. These enhanced responses were completely abolished, by a pretreatment of capsazepine, a selective antagonist of VR1 receptors, but not that by AM281, a selective antagonist of CB1 receptors. Based upon these results, our conclusion is that the anandamide enhances the pulmonary chemoreflex responses, this effect seems mediated through the activation of VR1 receptors.

Keywords: vanilloid type 1 receptor; pulmonary chemoreflex; sensitivity

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Bronchopulmonary vagal C-fiber afferents innervate all levels of the respiratory tract (1-2). Stimulation of these afferents elicits diffuse and pronounced cardiopulmonary reflex responses such as cough, bronchoconstriction, mucus secretion bradycardia and hypotension (3-6). The excitability of pulmonary C-fiber afferents can be markedly elevated by certain mediators (e.g., cyclooxygenase metabolites) released under pulmonary inflammation, which in turn may contribute to the pathogenesis of pulmonary inflammatory diseases such as asthma. However, the mechanisms underlying the mediator-induced sensitization of pulmonary C fibers are still poorly understood. The vanilloid type 1 receptor (VR1), a ligand-gated non-selective cation channel, is known to play a vital role in the inflammation-induced sensitization of nociceptors to various stimuli (7-10). Anandamide, an unstable arachidonate derivative, is released during tissue inflammation, is originally identified as an endogenous ligand of cannabinoid (CB) receptor (11) and is recently suggested to act as an endogenous ligand of VR1 receptors (12-14). Our experimental hypothesis is that anandamide might be the endogenous activator that sensitizes pulmonary C-fiber afferents, which consequently exaggerates the cardiopulmonary reflexes elicited by activation of pulmonary C fibers. To test this hypothesis, the present study were undertaken in a SD-rat model to investigate 1) whether anandamide enhances the C-fiber mediated cardiopulmonary response to phenyl biguanide (PBG), a selective stimulant of C fibers; and if yes, 2) whether this enhancing effect is only limited to PBG as a stimulator; and 3) what is the relative contribution of VR1 and CB1 receptors in this enhancing effect.

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Animal preparations

Male Sprague-Dawley rats (average

400 g) were initially anesthetized with an intraperitional injection of α-chloralose (~100 mg/kg; Sigma Chemical, St. Louis, MO, USA) and urethane (~500 mg/kg; Sigma) dissolved in a borax solution (2 %; Sigma); supplemental doses of the same anesthetics were given, whenever necessary, to maintain abolition of pain reflex elicited by paw-pinch. One femoral artery was cannulated for recording the arterial blood pressure with a pressure transducer. For right-atrial administration of pharmacological agents, the right jugular vein was cannulated and a catheter was advanced until its tip was positioned slightly above the right atrium. The volume of each bolus injection was 0.1 ml, which was first injected into the catheter (dead space ~ 0.2 ml) and then flushed into the circulation by an injection of 0.3 ml saline. A short tracheal cannula was inserted just below the larynx via a tracheostomy. Body temperature was maintained at ~36 ^{o}C throughout the experiment by means of a heating pad placed under the animal lying in a supine position. At the end of the experiment, the animal was killed by a right-atrial injection of KCl.

Measurement of cardiorespiratory responses

Rats breathed spontaneously via the tracheal cannula. Respiratory flow was measured with a heated pneumotachograph and a differential pressure transducer (Validyne MP45-14), and was integrated to give tidal volume. Ventilatory and cardiovascular signals were recorded on a polygraph (Gould RS 3200) and also on a videocassette recorder for later subsequent computer analysis. Before each injection, the lungs were hyperinflated (P_t > 10 cmH_2O) to establish a constant volume history.

Data analysis and statistical

Expiratory duration, respiratory frequency, tidal volume, heart rate and arterial blood pressure were analyzed on a breath-by-breath basis; these measurements

were made continuously for at least 10 breaths before (baseline) and 20 breaths after the injection of pharmacological agents. These parameters were analyzed using a computer equipped with an analog-to-digital converter (Gould DASA 4600) and a software (BioCybernatics 1.0). Results obtained from the computer analysis were routinely compared with those obtained by hand calculation for accuracy.

Data of were analyzed with a two-way ANOVA, unless otherwise mentioned. When the ANOVA showed a significant interaction, pair-wise comparisons were made with a *post hoc* analysis (Fisher's least significant difference). A P value < 0.05 were considered significant. Data are means ± S.E.M.

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Right-atrial injection of anandamide induced a consistent and pronounced enhancement of pulmonary chemoreflexes (Figs 1 and 2). This potentiating effect is not limited to PBG as a stimulant and is also found at the reflex responses induced by adenosine and lactic acid (Fig. 3). These enhanced responses were completely abolished, by a pretreatment of capsazepine, a selective blocker of VR1 receptors, but not that by AM281, a selective of CB1 receptors (Fig. 4). Based upon these results, our conclusion that the anandamide enhances the pulmonary chemoreflex responses, this effect seems mediated through the activation of VR1 receptors.

Whether anandamide-induced potentiating effect is resulted from its effect on hypersensitivity of pulmonary C-fiber is not known. However, it is well recognized that nociceptors, the counter part of pulmonary C-fiber nerve endings in the peripheral tissue, are sensitized during tissue inflammation (15-17); and the involvement of the endogenous activator of VR1 receptors is strongly suggested. In our previous studies (18), anandamide activates the VR1 receptor on pulmonary C-fiber

afferents. In addition, a recent report suggests that the involvement of VR1 receptor in the particulate matter-induced airway hyperreactivity and inflammation (19). Therefore, it is possible that, during pulmonary inflammation, anandamide may be the endogenous activator producing the VR1 receptor-mediated hypersensitivity of pulmonary C-fiber afferents. This hypothesis is further supported by the fact that, under certain inflammatory condition such as endotoxemia, the production of anandamide by macrophages is greatly increased; and this increase is involved in the cardiovascular responses (hypotension) under this pathophysiological condition (20-21). In addition, anandamide is one of the precursors of certain inflammatory mediators such as cyclooxygenase metabolites that are key mediators in the pathogenesis of lung inflammation. However, whether anandamide play a role in modulating the respiratory responses during pulmonary inflammation remained to be investigated.

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1. Paintal, A. S. Vagal sensory receptors and their reflex effects. *Physiol. Rev.* 53: 159-227, 1973.

2. Coleridge, J. C., and H. M. Coleridge. Afferent vagal C fibre innervation of the lungs and airways and its functional significance. *Rev. Physiol. Biochem. Pharmacol.* 99: 1-110, 1984

3. Paintal, A. S. Vagal sensory receptors and their reflex effects. *Physiol. Rev.* 53: 159-227, 1973.

4. Coleridge, J. C., and H. M. Coleridge. Afferent vagal C fibre innervation of the lungs and airways and its functional significance. *Rev. Physiol. Biochem. Pharmacol.* 99: 1-110, 1984.

5. Tatar, M., S. E. Webber, and J. G. Widdicombe. Lung C-fibre receptor activation and defensive reflexes in anaesthetized cats. *J. Physiol.* 402: 411-420, 1988.

6. Lee, L.-Y., and T. E. Pisarri. Afferent properties and reflex functions of bronchopulmonary C-fibers. *Respir. Physiol.* 125: 47-65, 2001.

7. Caterina, M. J., A. Leffler, A. B. Malmberg, W. J. Martin, J. Trafton, K. R. Petersen-Zeitz, M. Koltzenburg, A. I. Basbaum, and D. Julius. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 288: 306-313, 2000.

8. Davis, J. B., J. Gray, M. J. Gunthorpe, J. P. Hatcher, P. T. Davey, P. Overend, M. H. Harries, J. Latcham, C. Clapham, K. Atkinson, S. A. Hughes, K. Rance, E. Grau, A. J. Harper, P. L. Pugh, D. C. Rogers, S. Bingham, A. Randall, and S. A. Sheardown. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature* 405: 183-187, 2000.

9. Garcia-Martinez, C., M. Humet, R. Planells-Cases, A. Gomis, M. Caprini, F. Viana, E. De La Pena, F. Sanchez-Baeza, T. Carbonell, C. De

Felipe, E. Perez-Paya, C. Belmonte, A. Messeguer, and A. Ferrer-Montiel. Attenuation of thermal nociception and hyperalgesia by VR1 blockers. *Proc. Natl. Acad. Sci. USA* 99: 2374-2379, 2002.

10. Shin, J., H. Cho, S. W. Hwang, J. Jung, C. Y. Shin, S. Y. Lee, S. H. Kim, M. G. Lee, Y. H. Choi, J. Kim, N. A. Haber, D. B. Reichling, S. Khasar, J. D. Levine, and U. Oh. Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia. *Proc. Natl. Acad. Sci. USA* 99: 10150-10155, 2002.
11. Devane, W. A., L. Hanus, A. Breuer, R. G. Pertwee, L. A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, and R. Mechoulam. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258: 1946-1949, 1992.
12. Zygmunt, P. M., J. Petersson, D. A. Andersson, H. Chuang, M. Sorgard, V. Di Marzo, D. Julius, and E. D. Hogestatt. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* 400: 452-457, 1999.
13. Smart, D., M. J. Gunthorpe, J. C. Jerman, S. Nasir, J. Gray, A. I. Muir, J. K. Chambers, A. D. Randall, and J. B. Davis. The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). *Bri. J. Pharmacol.* 129: 227-230, 2000.
14. Smart, D., and J. C. Jerman. Anandamide: an endogenous activator of the vanilloid receptor. *Trends Pharmacol. Sci.* 21: 134, 2000.
15. Riedel, W., and G. Neeck. Nociception, pain, and antinociception: current concepts. *Z. Rheumatol*. 60: 404-415, 2001.
16. Kidd, B. L., and L. A. Urban. Mechanisms of inflammatory pain. *Br. J. Anaesth*. 87: 3-11, 2001.
17. Reeh, P. W., and G. Petho. Nociceptor

excitation by thermal sensitization--a hypothesis. *Prog. Brain Res.* 129: 39-50, 2000.

18. Lin, Y. S., and L.-Y. Lee. Stimulation of pulmonary vagal C-fibres by anandamide in anaesthetized rats: role of vanilloid type 1 receptors. *J. Physiol. (London)* 539: 947-955, 2002.

19. Veronesi, B., M. Oortgiesen, J. Roy, J. D. Carter, S. A. Simon, and S. H. Gavett. Vanilloid (capsaicin) receptors influence inflammatory sensitivity in response to particulate matter. *Toxicol. Appl. Pharmacol.* 169: 66-76, 2000.

20. Varga, K., J. A. Wagner, D. T. Bridgen, and G. Kunos. Platelet- and macrophage-derived endogenous cannabinoids are involved in endotoxin-induced hypotension. *FASEB J.* 12: 1035-1044, 1998.

21. Fowler, C. J., A. Stenstrom, and G. Tiger. Ibuprofen inhibits the metabolism of the endogenous cannabimimetic agent anandamide. *Pharmacol. Toxicol*. 80: 103-107, 1997.

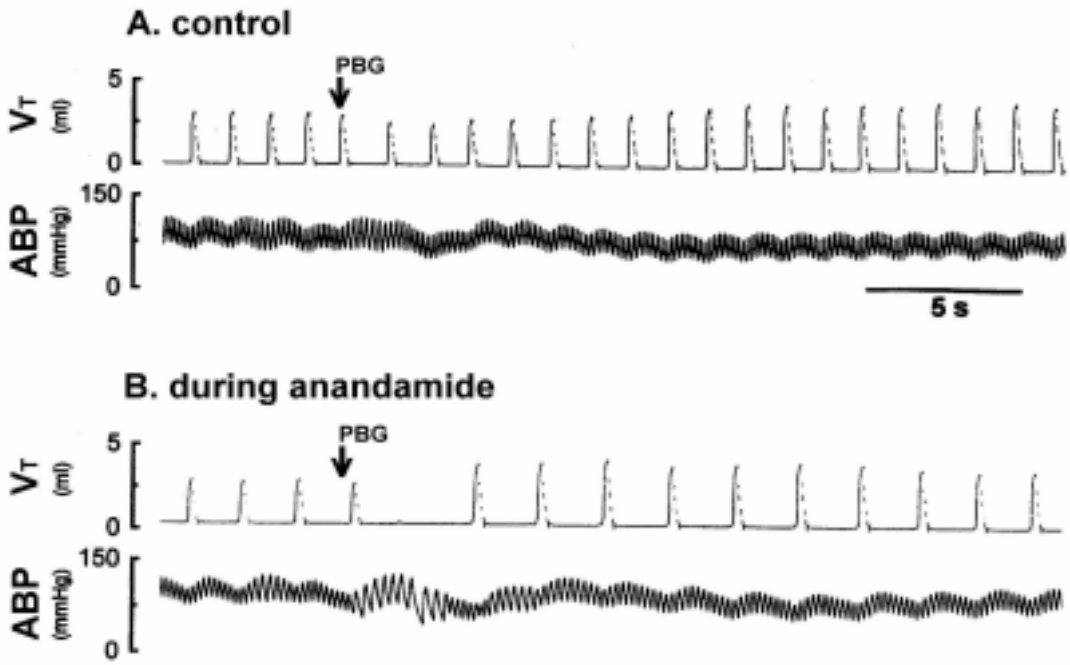

Fig. 1. Experimental records illustrating the effect of anandamide on pulmonary chemoreflexes elicited by right-atrial injections of phenyl biguanide (PBG, 3 μg/kg) in an anesthetized and spontaneously breathing rat (body weight: 320 g). Panel A: control response (before anandamide). Panel B: during anandamide: during an intravenous constant infusion of anandamide (0.5 mg/kg/min for 2 min). V_T, tidal volume; ABP, arterial blood pressure.

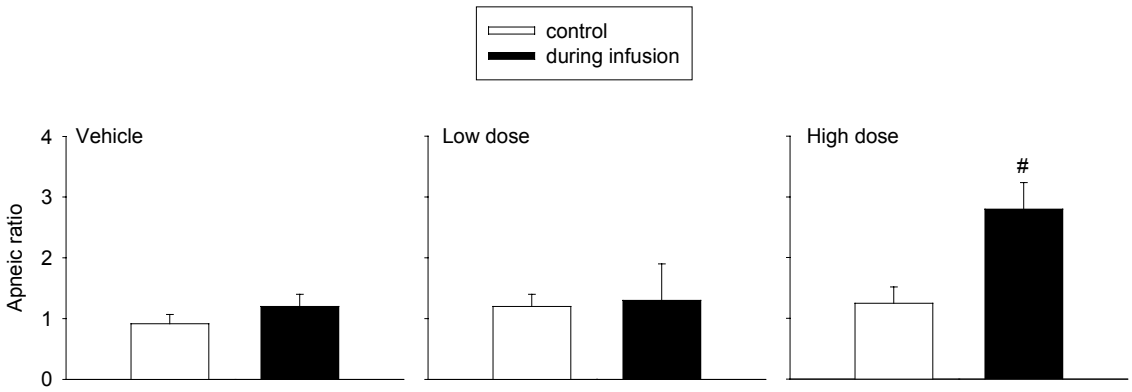

Fig. 2. Apneic responses induced by injection of phenyl biguanide (2-6 μg/kg) before and during infusion of three different doses of anandamide. Panels from left to right: vehicle (n = 6), low-dose (0.25 mg/kg/min; n = 6) and high-dose (0.5 mg/kg/min; n = 6) of anandamide, respectively. Open bar: control response (injection of PBG alone). Filled bar: response to PBG injection during infusion of anandamide or its vehicle. Apneic ratio: the longest expiratory duration (T_E) after the injection divided by the average T_E over 10 control breaths. Data are means ± SEM. #: significantly different from the control response.

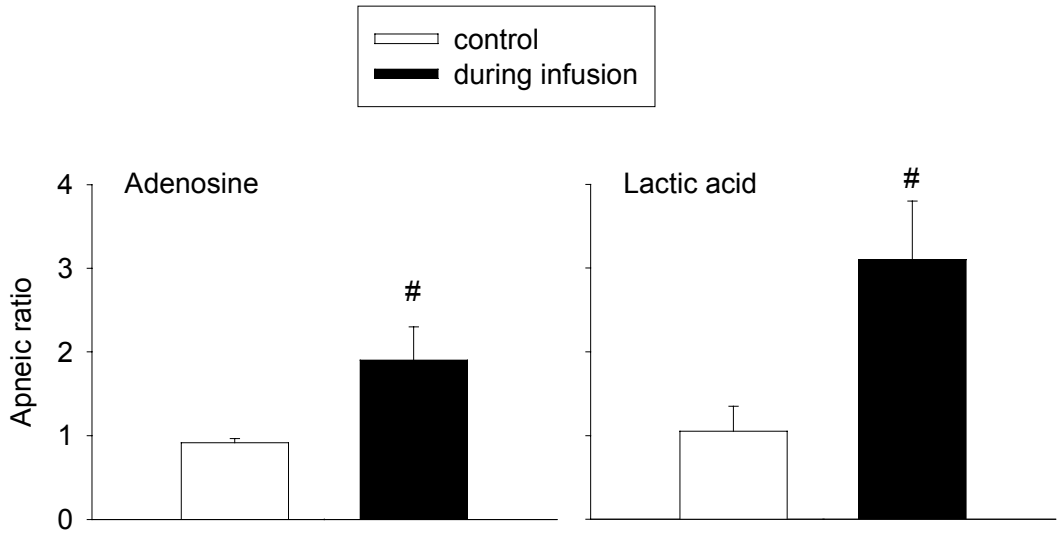

Fig. 3. Potentiating effect of anandamide on apneic responses to injection of adenosine (200 μg/kg; n = 6) and lactic acid (0.2 mmol/kg; n = 6). Open bar: control response. Filled bar: response to injection of adenosine or lactic acid during infusion. Data are means ± SEM. #: significantly different from the control response.

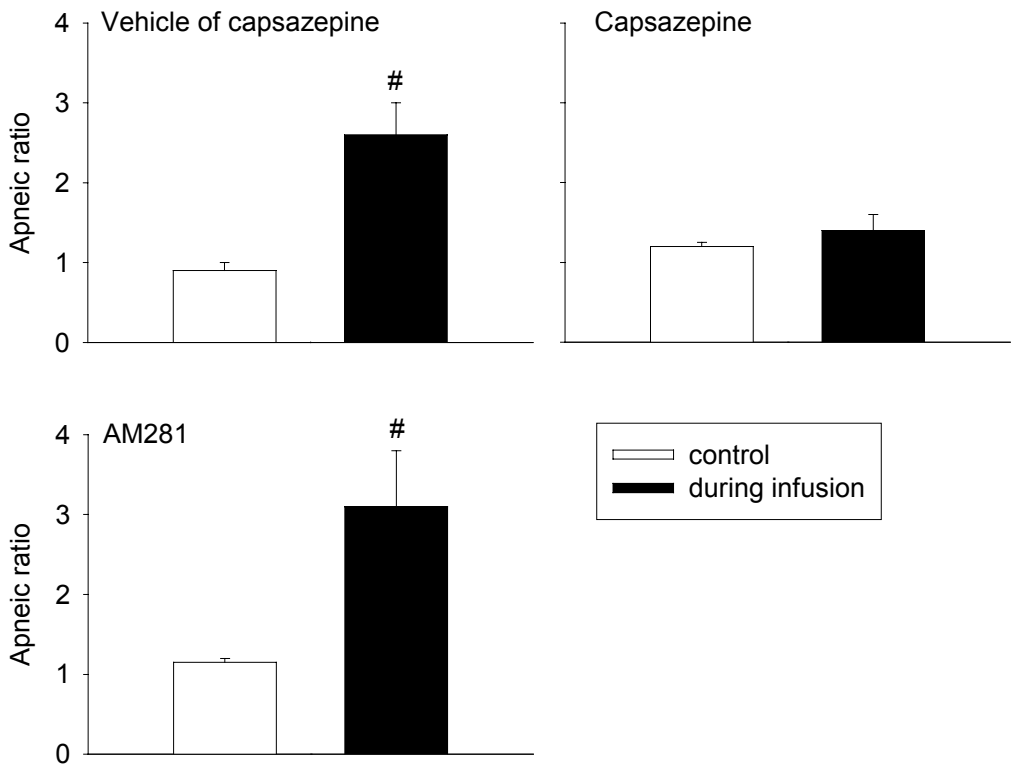

Fig. 4. Contribution of VR1 and CB1 receptors to the anandamide-induced enhancement of apneic responses to phenyl biguanide (PBG, 2-6 μg/kg). Upper panels: pretreatment with capsazepine (a selective antagonist of VR1 receptors; 3 mg/kg; n = 6) or its vehicle (n = 6). Lower panel: pretreatment with AM281 (a selective antagonist of CB1 receptors; 0.3 mg/kg; n = 6). Open bar: control response (PBG along). Filled bar: response to PBG injection during infusion of anandamide. Data are means ± SEM. #: significantly different from the control response.