Bradykinin-Induced Airway Contraction in Two Lines of Guinea Pigs with Congenitally Different Airway Sensitivity

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ABSTRACT. The airway responsiveness to bradykinin (0.1, 1 and 10 μg/kg, i.v.) was examined in two lines of guinea pigs, BHS (bronchial hypersensitive) and BHR (bronchial hypo-sensitive) lines, with different airway sensitivity to inhalation of acetylcholine (ACh)-aerosol. Normal Hartley strain guinea pigs were used as a control group. The airway contraction was measured by recording intratracheal pressure (P_{IT}) and respiratory airflow (V) under the condition of artificial ventilation in anesthetized guinea pigs. The results show airway responsiveness to bradykinin in BHS guinea pigs to be significantly greater than in BHR and normal Hartley strain guinea pigs. — KEY words: bradykinin, bronchoconstriction, guinea pig.


Two lines of guinea pigs with different airway sensitivity have been developed by genetic selection on responsiveness to ACh and histamine aerosols. They are the BHS line which is bronchial hypersensitive, and the BHR line which is bronchial hypo-sensitive to these bronchoconstrictive agents [5]. At present, these lines have been bred to the F19-generation at the Institute of Bio-Active Science, Nippon Zoki Pharmaceutical Co., Ltd. A previous study demonstrated an obvious difference in airway responsiveness between the two lines at their F4-F7-generations, when they inhaled or were injected with ACh, histamine and leukotriene D4 (LTD4) [6]. Although the BHS line has a greater number of muscarinic receptors of the lung tissue and a higher binding affinity compared to those of BHR line [6], the basic mechanisms of the differences in airway sensitivity have not been elucidated. The mechanisms might be expected to involve intracellular metabolism in the smooth muscle and neural and humoral regulation of the smooth muscle contraction.

The present study was aimed at elucidating whether the difference in airway responsiveness between the two lines is present to bradykinin which is a potent bronchoconstrictive agent in animals and humans, and can stimulate C-fiber endings in the airway of some species such as guinea pigs [3].

BHS (600.0 ± 29.9 g, mean ± SE, n=8), BHR (603.8 ± 26.8 g, mean ± SE, n=8) and normal Hartley guinea pigs (582.5 ± 21.5 g, mean ± SE, n=8; provided by Saitama Experimental Animals Supply Co., Ltd.) were used in this experiment, respectively. They were systemically anesthetized by intraperitoneal injection of urethane (1 g/kg). A tracheal cannula was inserted into the lower trachea, through an incision of the cervical trachea, and connected to a positive pressure-ventilator. An absolute pressure-transducer (Toyoda, PD104) and a differential pressure-transducer (Toyoda, DD102S) were connected to the line to the tracheal cannula in order to measure the intratracheal pressure (P_{IT}) and respiratory airflow (V) changes induced by bradykinin injection during artificial ventilation.

The artificial ventilation was done under the condition of 70 cycles/min of ventilatory frequency, and the initial P_{IT} level was adjusted to between 1.02 and 1.27 kPa (average) before the bradykinin injection. In all animals, the thorax was opened and the expiratory line in the ventilator was loaded at 0.15 kPa by immersing the outlet tube into a water bath to protect the lung from collapsing.

A fine cannula was inserted into the right external jugular vein to inject bradykinin which was diluted with saline. The dose of bradykinin injected was 0.1, 1 and 10 μg/kg, and in each animal a bolus of bradykinin solution was administered during approximately 10 sec from the lowest to highest dose at 20 min intervals. The P_{IT} and V were recorded 1 min before the bradykinin injection and for at least 3 min after the injection. If the P_{IT} did not recover to the pre-injection level due to bronchoconstriction induced by the drug injection, instantaneous hyperinflation of the lung was performed by occluding the outlet of the ventilator to maintain the patency of the peripheral small airway.

Statistical tests were applied to the raw data. Student’s unpaired t test was used for comparisons between two animal groups, and two-way analysis of variance (ANOVA) was used to analyse dose-response in the same animal group.

An example of recordings of P_{IT} and V changes induced by bradykinin injection (0.1–10 μg/kg) is shown in Fig. 1. Increases in P_{IT} and decreases in V were found after the injection at doses of 1 and 10 μg/kg in both BHS and BHR guinea pigs, while much weaker changes were observed at 0.1 μg/kg in BHS guinea pigs only. In the present study, the changes in P_{IT} were more marked than the changes in V, therefore the following description is confined to the changes in P_{IT}. The maximum increase in P_{IT} was observed at 40.3 ± 2.4 sec and 35.5 ± 1.9 sec after the onset of injection at 1 and 10 μg/kg, respectively. The increased P_{IT} was sustained in all BHS guinea pigs, showing 120 to 155% of pre-injection levels at three minutes after the injection at 1 and 10 μg/kg. In all BHR guinea pigs, however, P_{IT} recovered almost to the pre-injection level after the 1 μg/kg
The maximum \( P_{tr} \) response at all doses of bradykinin was compared among the BHS, BHR and normal Hartley guinea pigs, and the results are shown in Fig. 2. All three animal groups showed a dose-dependent increase in the maximum \( P_{tr} \) \((P<0.01)\). The maximum \( P_{tr} \) response in BHS guinea pigs was greater than that of the BHR and normal Hartley guinea pigs, and statistically significant differences were found at 0.1 \( \mu g \) \( (P<0.01) \), 1 \( \mu g \) \( (P<0.05) \) and at 10 \( \mu g \) per kg \( (P<0.05) \). The values of maximum \( P_{tr} \) in BHS guinea pigs were 1.1 \( \pm 0.04 \), 1.75 \( \pm 0.21 \) and 2.38 \( \pm 0.35 \) kPa at 0.1, 1 and 10 \( \mu g \) per kg respectively, and were 108.9, 176.2 and 248.3\% of the respective pre-injection levels. There were no statistical differences in the \( P_{tr} \) values between the BHR and normal Hartley guinea pigs at any dose.

The present study demonstrates that a clear difference in magnitude of airway contraction to bradykinin is present between the BHS and BHR or normal Hartley guinea pigs. The mechanisms related to such a difference are conceived to be complex since the airway hyperresponsiveness is influenced by various physiological factors, e.g., the number or affinity of receptors bound by ligands and intracellular metabolism in the smooth muscle, neural regulations by the autonomic nerves and by axonal reflex mechanisms of C-fibers, chemical mediators and enzymes such as neutral endopeptidase and angiotensin-converting enzyme in the airway and the lung [1].

The guinea pig's airway and lung tissue have bradykinin \( B_2 \) receptor and possibly \( B_3 \) receptor which induce potent bronchoconstriction [2, 9]. In the guinea pig, an electrophysiological study demonstrated that bradykinin can potently stimulate the C-fibers, but not A-delta fibers, by binding to the \( B_2 \) receptor [3], indicating a possibility that the axon reflex by C-fibers can mediate the airway contraction. In addition, bradykinin induced different cytokines such as interleukin from the isolated lung tissue [4]. Therefore, it is necessary to consider that the bradykinin induced airway contraction involves the stimulating mechanisms of the vagal afferent pathway, especially C-fibers, as well as the contribution of chemical mediators. A recent histological study [7] showed that, compared with BHR guinea pigs, the number per unit area \( (N_u) \) and the

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**Fig. 1.** Changes in intratracheal pressure \( (P_{tr}) \) and respiratory airflow \( (\dot{V}) \) after intravenous injection of bradykinin in a bronchial hypersensitive (BHS) and a bronchial hyporesponsive (BHR) guinea pig. Bradykinin (0.1 \( \mu g \), 1 \( \mu g \), 10 \( \mu g \) per kg) was injected at the rectangular mark in the record.

**Fig. 2.** Maximum \( P_{tr} \) responses after bradykinin injections in BHS, BHR and normal Hartley guinea pigs. *: \( P<0.05 \), **: \( P<0.01 \) vs. BHR (Student's unpaired t-test)
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total length per unit area ($L_n$) of AChE-positive nerves in the lamina propria and smooth muscle layer of the peripheral airway was significantly greater in BHS guinea pigs. In addition, BHS guinea pigs showed a tendency to have greater $N_A$ and $L_n$ of tyrosine hydroxylase (TH), calcitonin gene-related peptide (CGRP) and substance P (SP) immunoreactive nerve fibers. This evidence suggests a possibility that in BHS guinea pigs neural regulation plays a more important role on smooth muscle contraction than in BHR guinea pigs. In order to demonstrate this possibility, further studies including experiments with aerosol-inhalations or direct instillation of bradykinin will be required, since the intra-airway application of bradykinin can stimulate the afferent fibers and release sensory neuropeptides in the mucus membrane of the airway [4].

The lack of a significant difference in the airway responsiveness to bradykinin between BHR and normal Hartley guinea pigs may suggest that the sensitivity of nerves and airway smooth muscles to bradykinin is similar in these guinea pigs. It is of great interest to note that the BHS guinea pig has higher sensitivity to not only cholinergic agents but also non-cholinergic agents such as bradykinin, used in the present study, and leukotriene $\Delta_2$, used in a previous study [6], since these findings might assure that this guinea pig is a useful model animal for extensive investigation of the basic mechanisms of airway hypersensitivity.

REFERENCES