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European Journal of Pharmacology 359 (1998) 171–176

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Elastase in hyperpnea-induced guinea pig airway constriction

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Received 25 August 1998; accepted 28 August 1998

Abstract

Aerosolized elastase has been shown to produce airway constriction in guinea pigs. In this study, we examined whether endogenous elastase plays a role in isocapnic hyperpnea-induced airway constriction using an elastase inhibitor, eglin-c. The study was divided into three experiments. In the first experiment, we used an elastase inhibitor, eglin-c, to suppress hyperpnea-induced bronchoconstriction. Twenty-two young male Hartley guinea pigs were divided into three groups: control ($n = 8$), eglin-c(1) (a lower dose of eglin-c, $n = 7$), and eglin-c(2) (a higher dose of eglin-c, $n = 7$). In the second experiment, we tested whether eglin-c affects pulmonary function following 15 min of normal air ventilation in two groups of animals: control ($n = 8$) and eglin-c ($n = 8$). In the third experiment, animals were divided into two groups: control ($n = 7$) and compound 48/80 (a mast cell degranulating agent, $n = 7$). Airway function was examined in the anesthetized–paralyzed animal. In the first and third experiments, 15 min of isocapnic hyperpnea caused marked decreases in dynamic respiratory compliance, forced expiratory flow at 0.1 s and maximal expiratory flow at 50% total lung capacity, demonstrating hyperpnea-induced airway constriction. This bronchoconstriction was significantly attenuated by eglin-c and by pretreatment with compound 48/80. In the second experiment, eglin-c did not significantly affect bronchial function following normal air ventilation. These data suggest that elastase released from mast cells directly or indirectly induces hyperpnea-induced bronchoconstriction.

Keywords: Bronchial reactivity; Eglin-c; Mast cell; Mediator

1. Introduction

A short period of isocapnic hyperventilation results in airway constriction in guinea pigs (Ray et al., 1989; Fang and Lai, 1993). This hyperpnea-induced bronchoconstriction is closely related to water and heat loss during hyperventilation. In addition to the loss of water and heat, hyperpnea with dry air causes mucosal damage in airways, resulting in the degranulation of mast cells (Pliss et al., 1990). Mediators and elastase are then released from mast cells (Caughey, 1994; Doran et al., 1994).

Suzuki et al. (1996b) found that exogenous elastase induced airway constriction in vivo and this constriction was prevented by elimination of elastase activity (Suzuki et al., 1996a). It is not clear whether endogenous elastase plays a role in hyperpnea-induced airway constriction in guinea pigs. We demonstrated previously that eglin-c, an inhibitor of serine elastase and cathepsin G (Schnebli, 1987), suppressed endogenous elastase activity generated in the lungs (Lai and Diamond, 1990). In addition, we used eglin-c to inhibit monocrotaline-initiated, elastate-related airway constriction (Lai and Zhou, 1997). If endogenous elastase causes hyperpnea-induced bronchoconstriction, we hypothesized that eglin-c can attenuate or prevent this type of airway constriction. To test this hypothesis, eglin-c was intratracheally instilled to inhibit endogenous elastase in the lung of guinea pigs, and to see if eglin-c attenuates hyperpnea-induced bronchoconstriction.

2. Materials and methods

The study was conducted according to the Guidelines of the American Physiology Society and was approved by the Animal Care and Use Committee of the National Taiwan University.

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0014-2999/98/$ - see front matter
PII: S0014-2999(98)00646-3

2.1. Preparation of experimental animals

This study was divided into three experiments. In the first experiment, we used an elastase inhibitor, eglin-c, to inhibit hyperpnea-induced airway constriction. Twenty-two young Hartley guinea pigs weighing 295 ± 31 g were divided into three groups: control ($n = 8$), eglin-c(1) ($n = 7$), and eglin-c(2) ($n = 7$). Following anesthesia with sodium pentobarbital (30–40 mg/kg), the trachea, carotid artery and jugular vein were cannulated. After being paralyzed with gallamine triethiodide (4 mg/kg), the animals were artificially ventilated and were intravenously injected with propranolol (0.5 mg/kg) to block sympathoadrenal effects. Subsequently, 0.25 ml of either saline (the control group) or eglin-c solution was intratracheally instilled. The eglin-c-treated animals were divided into low-dose (eglin-c(1), 37.5 mg/kg) and high-dose (eglin-c(2), 55 mg/kg) groups. These doses of eglin-c were those used in our previous papers (Lai and Diamond, 1990; Lai and Zhou, 1997). Airway function was assessed 10 min after the intratracheal instillation of saline or eglin-c (the baseline period) as well as 5 min after the 15-min period of hyperventilation (the recovery period).

In the second experiment, we tested whether eglin-c affects pulmonary function during normal air ventilation. Guinea pigs were divided into two groups: control ($n = 8$) and eglin-c ($n = 8$). Anesthetized–paralyzed animals were prepared according to the above methods. Ten minutes after propranolol administration, 0.25 ml of saline (the control group) or eglin-c (55 mg/kg) was intratracheally instilled. Bronchial function was tested 10 min (the baseline period) and 30 min (equivalent to the recovery period) after the intratracheal instillation of saline or eglin-c.

We used a mast cell degranulating agent, compound 48/80, to induce degranulation of mast cells in the third experiment of this study. Guinea pigs were divided into two groups: control ($n = 7$) and compound 48/80 ($n = 7$). Animals were subcutaneously injected with saline (the control group) or compound 48/80 for 3 days. According to the methods described by Saria et al. (1984), the total dosage of compound 48/80 was 25 mg/kg. Three consecutive daily doses of compound 48/80 were 8, 8, and 9 mg/kg. Usually the daily dose was divided into two injections for each animal. Anesthetized–paralyzed animals were prepared according to the above methods. Airway function was assessed 10 min after the intravenous injection of propranolol (the baseline period) as well as 5 min after the 15-min period of isocapnic hyperventilation (the recovery period).

On the day of the study, the anesthetized–paralyzed animals were artificially ventilated, and bronchial function was assessed before and after hyperpnea. The experimental protocol was carried out according to our previous study (Fang and Lai, 1993). The basic sequence for each experiment was baseline, hyperpnea, and recovery. During the baseline period, each animal was ventilated with humidified air (tidal volume, 6 ml/kg; frequency, 60 breaths/min) at room temperature. Then it was hyperventilated for 15 min with a dry 95% O_2–5% CO_2 gas mixture at room temperature (tidal volume, 10 ml/kg; frequency, 150 breaths/min). Subsequently, each animal was ventilated with humidified air, the same as that during the baseline period, for 20 min (the recovery period). We tested bronchial function during the baseline and the recovery periods.

2.2. Evaluation of bronchial function

During the baseline period, we first performed the full maximal expiratory flow–volume maneuver 2–3 times to obtain baseline total lung capacity (lung volume at airway opening pressure = 30 cmH_2O). Subsequently, partial maximal expiratory flow–volume maneuvers were carried

Table 1
Body weight and baseline respiratory variables of guinea pigs

Group	n	Body weight (g)	TLC (ml)	FRC (ml)	$\dot{V}_{max50}$ (ml/s)	$FEV_{0.1}$ (ml)	Crs (ml/cmH_2O)
First experiment							
Control	8	324 ± 52	7.92 ± 0.53	3.07 ± 0.22	23.1 ± 5.6	2.46 ± 0.34	0.15 ± 0.01
Eglin-c(1)	8	260 ± 13	7.80 ± 0.21	3.02 ± 0.20	22.3 ± 4.5	1.91 ± 0.26	0.12 ± 0.01
Eglin c(2)	8	299 ± 29	8.20 ± 0.35	3.34 ± 0.24	17.9 ± 3.4	2.16 ± 0.26	0.14 ± 0.01
Second experiment							
Control	8	425 ± 27	8.64 ± 0.59	3.30 ± 0.10	33.3 ± 6.5	3.07 ± 0.29	0.18 ± 0.02
Eglin-c	8	338 ± 23[a]	7.96 ± 0.31	3.15 ± 0.14	23.0 ± 3.1	2.29 ± 0.16[a]	0.13 ± 0.01
Third experiment							
Control	7	343 ± 4	10.3 ± 0.6	3.77 ± 0.36	54.3 ± 6.8	3.71 ± 0.25	0.28 ± 0.01
Compound 48/80	7	322 ± 15	10.1 ± 0.3	3.59 ± 0.14	52.1 ± 6.5	3.25 ± 0.33	0.18 ± 0.03[a]

Values are means ± S.E.
n, number of animals; TLC, total lung capacity; FRC, functional residual capacity; $\dot{V}_{max50}$, maximal expiratory flow rate at 50% TLC; $FEV_{0.1}$, forced expiratory volume at 0.1 s; Crs, dynamic respiratory compliance.
[a]Significant difference ($P < 0.05$) compared to the respective control group.

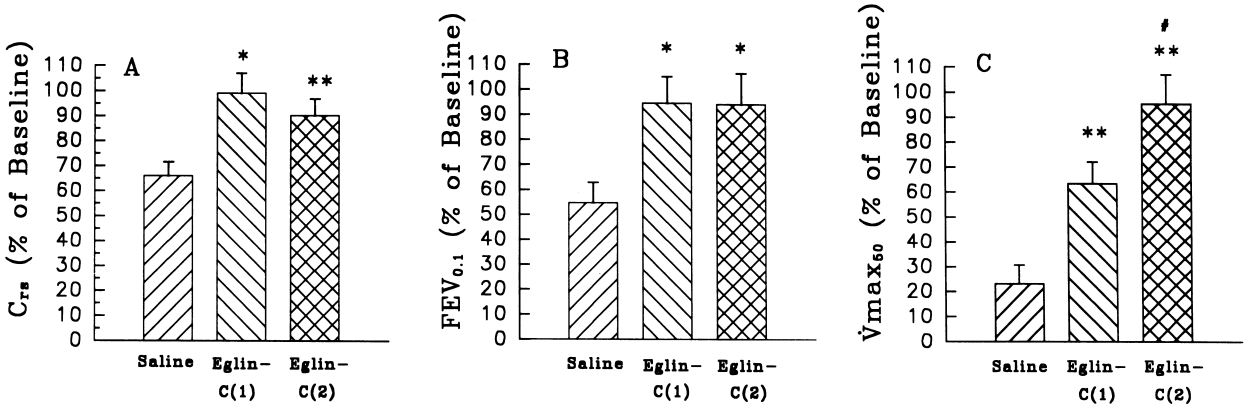

Fig. 1. Effects of eglin-c on isocapnic hyperpnea-induced decreases in dynamic respiratory compliance (Crs) (A), forced expiratory volume at 0.1 s ($FEV_{0.1}$) (B), and maximal expiratory flow at 50% total lung capacity ($\dot{V}_{max50}$) (C). Eglin-c(1) was given at a low dose (37.5 mg/kg) while eglin-c(2) was given at a high dose (55 mg/kg). Significant differences between the saline control and eglin-c groups: $*\ P < 0.05$ and $**\ P < 0.01$. $^{\#}$Significant difference ($P < 0.05$) between the two eglin-c groups.

out before and after hyperpnea to examine the hyperpnea-induced bronchoconstriction. Since large-volume inflation of the lungs may ameliorate airway constriction (Loring et al., 1981; Ray et al., 1991), the partial maximal expiratory flow–volume maneuver was used to avoid the abolishment of hyperpnea-induced bronchoconstriction. The partial maximal expiratory flow–volume maneuver was a modification of a previously reported method (Lai, 1988). Each anesthetized animal was placed supine inside a whole-body plethysmograph. The flow rate was monitored with a Validyne DP45 differential pressure transducer as the pressure drop across three layers of 325-mesh wire screen in the wall of the plethysmograph. The lung volume change was determined by integration of flow. The airway opening pressure (Pao) was measured with a Statham PM 131 pressure transducer. The lungs were inflated three times to a lung volume such that Pao = 10 cmH_2O (instead of the usual Pao = 30 cmH_2O for the full maximal expiratory flow–volume maneuver). At peak volume during the third inflation, the inflation valve was shut off and immediately another solenoid valve for deflation was automatically turned on. The deflation valve was connected to a 20-l container with a negative pressure of 40 cm H_2O. This negative pressure produced the maximal expiratory flow ($\dot{V}_{max}$). The changes in flow, volume and Pao were traced on a polygraph (Gould, model TA11) and the partial maximal expiratory flow–volume plot was also stored on a Cathode Ray Storage Oscilloscope (VC-6025, Hitachi). During artificial ventilation (between the interval of the partial maximal expiratory flow–volume maneuvers), tidal volume (V_T) and its accompanying airway opening pressure difference (ΔPao) were used to calculate dynamic respiratory compliance ($Crs = V_T/\Delta Pao$). The difference in airway opening pressure was measured between end-inspiration and end-expiration (i.e., instances of no flow). Crs, forced expiratory volume at 0.1 s ($FEV_{0.1}$), and $\dot{V}_{max}$ at 50% baseline total lung capacity ($\dot{V}_{max50}$) are measures of airway resistance and changes in these variables may

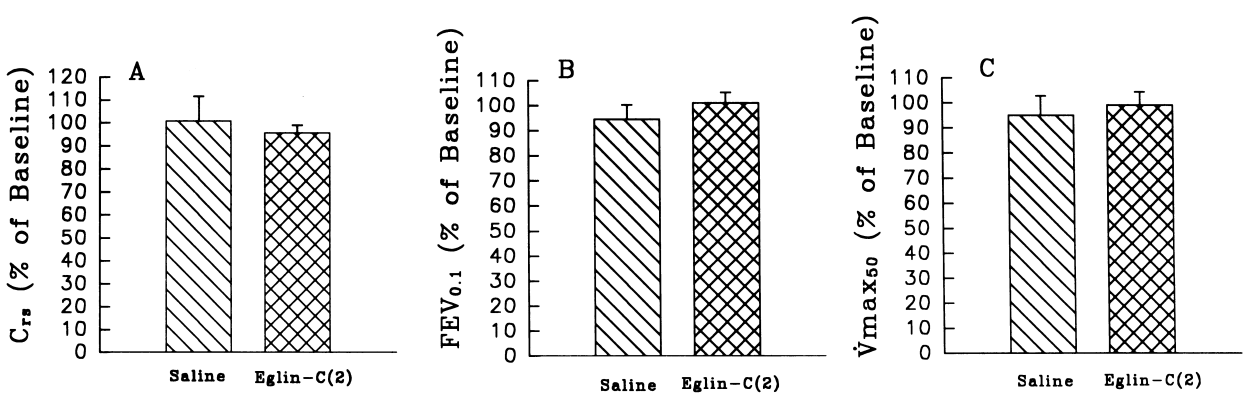

Fig. 2. Effects of eglin-c on dynamic respiratory compliance (Crs) (A), forced expiratory volume at 0.1 s ($FEV_{0.1}$) (B), and maximal expiratory flow at 50% total lung capacity ($\dot{V}_{max50}$) (C) during ventilation with normal air.

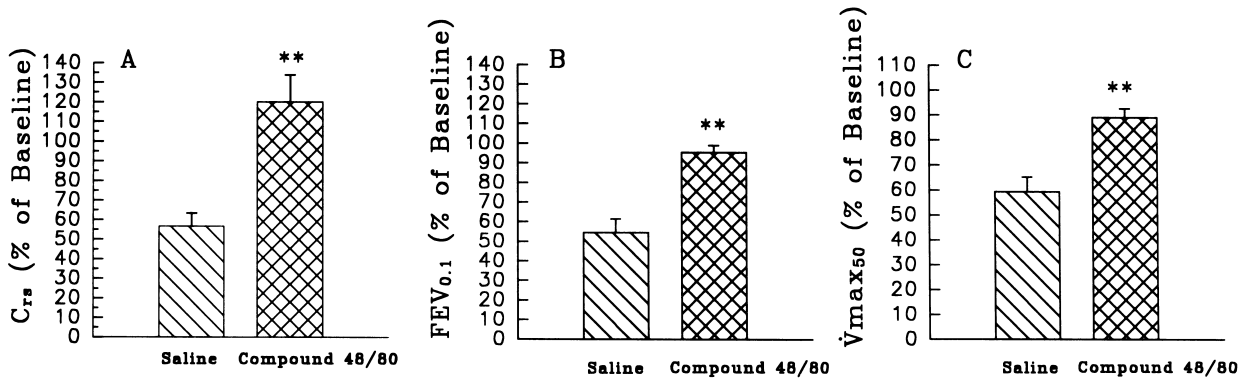

Fig. 3. Effects of compound 48/80 on isocapnic hyperpnea-induced decreases in dynamic respiratory compliance (Crs) (A), forced expiratory volume at 0.1 s ($FEV_{0.1}$) (B), and maximal expiratory flow at 50% total lung capacity ($\dot{V}_{max50}$) (C). ** Significant differences ($P < 0.01$) compared to the saline control group.

indicate bronchoconstriction. The general experimental procedure consisted of obtaining the values of Crs, $FEV_{0.1}$ and $\dot{V}_{max50}$ before and 5 min after the 15-min period of hyperpnea.

2.3. Statistical analysis

All values are reported as means ± S.E. Analysis of variance was used to establish the statistical significance of differences among groups. If significant differences among groups were detected by using the analysis of variance, Duncan's multiple range test was used to determine differences between groups. Differences were considered significant if $P < 0.05$.

3. Results

Body weight and baseline respiratory variables for the first experiment are listed in Table 1. There were no significant differences in body weight or respiratory variables between the groups. In the control group, isocapnic hyperpnea induced marked decreases in Crs, $FEV_{0.1}$ and $\dot{V}_{max50}$, indicating hyperpnea-induced airway constriction. This bronchoconstriction was significantly attenuated by either eglin-c(1) (37.5 mg/kg) or eglin-c(2) (55 mg/kg) (Fig. 1).

In the second experiment, animals in the control group were heavier than animals in the experimental group (Table 1). However, no significant differences in baseline respiratory variables (in terms of absolute value or per kg body weight) were found between the groups. Following 15 min of normal air ventilation, no significant decreases in either Crs, $FEV_{0.1}$ or $\dot{V}_{max50}$ were found in either the control or the eglin-c group (Fig. 2).

No significant differences in either body weight or baseline respiratory variables between groups were found in the third experiment, except that Crs was lower in the compound 48/80 group (Table 1). Hyperpnea (15 min) caused marked decreases in Crs, $FEV_{0.1}$ and $\dot{V}_{max50}$ in the control group, but not in the compound 48/80 group (Fig. 3). Changes in Crs, $FEV_{0.1}$ and $\dot{V}_{max50}$ were significantly different between these two groups.

4. Discussion

4.1. Elastase and hyperpnea-induced airway constriction

Similar to our previous studies (Fang and Lai, 1993), we found that hyperpnea induced airway constriction in saline controls. The airway constriction was significantly attenuated by the administration of an elastase inhibitor, eglin-c. Therefore, we showed that endogenous elastase plays an important role in hyperpnea-induced airway constriction. It is possible that elastase acts in two ways to augment hyperpnea-induced airway constriction: a direct bronchial constricting action and an indirect action via the release of bronchoconstricting mediators. Suzuki et al. (1996b) demonstrated that aerosolized elastase caused airway constriction, an effect which was prevented by elastase inhibitor or by removal of the enzymatic activity from elastase (Suzuki et al., 1996a). Similar to Suzuki et al. (1996b), we found that eglin-c inhibited hyperpnea-induced airway constriction in this study. We administered sufficient dosages of eglin-c in our study because a higher dose of eglin-c did not produced a greater inhibition of hyperpnea-induced airway constriction (Fig. 1). Besides, eglin-c had no effect on bronchial function during normal artificial ventilation (Fig. 2). It seems, therefore, that eglin-c exerted its bronchial effect only when there was an elevated level of elastase. Furthermore, it is known that elastase can release mediators from inflammatory cells. In addition to a possible direct constricting effect of elastase, serine elastase can enhance the release of chemical mediators. For example, serine proteinases augment the release of histamine in sheep (Molinari et al., 1996), rats (Emadi-Khiav and Pearce, 1994) and humans (Hultsch et al., 1988). It is interesting to mention that histamine released

in this way causes bronchoconstriction in vivo in sheep (Molinari et al., 1996).

The source of endogenous elastase is speculative. It is possible that hyperpnea induces mucosal injury and mast cell degranulation in airways, which results in the release of elastase. Following hyperpnea with dry air, there is usually an increase in the number of epithelial cells in bronchoalveolar fluid (Freed et al., 1987; Ingenito et al., 1990; Pliss et al., 1990). This loss of epithelial cells from airway surfaces is indicative of mucosal injury in the airways. This injury may result in mast cell degranulation which, in turn, releases elastase and mediators. Two lines of evidence support our idea that mast cells are the source of endogenous proteinases and mediators. First, after the pretreatment with compound 48/80, 15 min of isocapnic hyperpnea did not induce airway constriction (Fig. 3). This implies that prior depletion of elastase and mediators by compound 48/80 can prevent hyperpnea-induced bronchoconstriction (Rafferty et al., 1985). Second, Omori et al. (1995) found that mast cell degranulation occurs either during or immediately after hyperpnea with dry air.

4.2. Elastase and mediators of hyperpnea-induced bronchoconstriction

In guinea pigs, there are two main mediators of hyperpnea-induced bronchoconstriction: tachykinins (Ray et al., 1989; Solway et al., 1993) and leukotrienes (Garland et al., 1993). After its release from mast cells, histamine can induce the release of tachykinins (Saria et al., 1988) which, in turn, enhance the release of histamine (Joos and Pauwels, 1993). Leukotrienes are released directly from mast cells during degranulation (Doran et al., 1994).

In addition to acting as a mast cell secretagogue, compound 48/80 can also activate afferent C-fibers and release tachykinins in the lungs. Mapp et al. (1993) found that compound 48/80 caused airway constriction, an effect which was significantly attenuated by capsaicin pretreatment. Mapp et al. (1993) also found that pretreatment with compound 48/80 significantly attenuated toluene diisocyanate-induced airway constriction. In addition, this attenuation by compound 48/80 was significantly blocked by a tachykinin NK_2 receptor antagonist, MEN 10,207. Similarly, Saria et al. (1984) showed that compound 48/80-induced vascular protein leakage could be inhibited by capsaicin pretreatment and by a substance P receptor antagonist. According to the above two studies, it is apparent that compound 48/80 activates afferent C-fibers in the lungs, which release tachykinins. Subsequently, tachykinins cause airway constriction and vascular protein leakage via tachykinin NK_2 and NK_1 receptors, respectively. After chronic pretreatment with compound 48/80, the secretagogue should have produced both degranulation of mast cells and activation (and thus depletion) of afferent C-fibers. Therefore, the lack of hyperpnea-induced airway constriction in animals pretreated with compound 48–80 could be explained by a depletion of mediators (such as leukotrienes) in mast cells and/or a depletion of tachykinins in afferent C-fibers. Accordingly, the mechanism of the mast cell involvement is unclear and we cannot exclude other mechanisms in addition to those discussed.

In summary, we found that eglin-c and pretreatment with compound 48/80 attenuated hyperpnea-induced airway constriction. Our and other investigators' (Freed et al., 1987) data suggest that hyperpnea causes mucosal damage and mast cell degranulation. Elastase released from mast cells may induce hyperpnea-induced airway constriction directly or indirectly via the release of other mediators such as tachykinins and leukotrienes. Our results support the idea that endogenous elastase plays an important contributing role in hyperpnea-induced airway constriction.

Acknowledgements

We are grateful to Dr. Hans Peter Schnebli, Ciba-Geigy Research Department, Basel, Switzerland for providing eglin-c for this study. We thank Miss C.L. Huang and Mr. F.Y. Ko for their technical assistance. This investigation was supported by the National Science Council (NSC86-2314-B-002-186 and NSC86-2314-B-002-011M29).

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