

Adrenal Influences on the Inhibitory Effects of Procaterol, a Selective Beta-Two-Adrenoceptor Agonist, on Antigen-Induced Airway Microvascular Leakage and Bronchoconstriction in Guinea Pigs

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Key Words

Antigen · Asthma · β -Adrenoceptor · Bronchoconstriction · Epinephrine · Microvascular leakage · Plasma exudation · Procaterol

Abstract

While the guinea pig has been the preferred choice for use as a model of allergic bronchial asthma in the evaluation of anti-asthmatic drugs, it has been shown that antigen-induced bronchoconstriction in guinea pigs is attenuated by epinephrine released from the adrenal gland. In order to investigate the possible influence of the adrenal gland on the effects of antiexudative and bronchodilative drugs on antigen-induced airway responses, we examined the inhibitory effects of procaterol, a selective β_2 -adrenoceptor agonist, on antigen-induced airway microvascular leakage and bronchoconstriction in adrenalectomized guinea pigs and compared them with the drug's effects in sham-operated animals. Guinea pigs sensitized passively with anti-ovalbumin (OA) guinea-pig serum were adrenalectomized or sham-operated under urethane anesthesia and examined 30 min after surgery in the following experiments. (1) Animals were intravenously administered Evans blue dye to quantify airway plasma exudation, and then OA was in-

haled for 10 min while measuring pulmonary inflation pressure, a parameter of bronchoconstriction. Procaterol (1, 3, 10, or 30 μ g/kg) or saline (control) was administered into the airways 10 min prior to OA inhalation. The amount of extravasated Evans blue dye in the airways was calculated. (2) Venous blood samples were collected during OA or saline inhalation and plasma catecholamine levels were compared. In control animals, OA-induced increases in both the amount of Evans blue dye and in pulmonary inflation pressure were markedly greater in adrenalectomized animals than in sham-operated animals. Procaterol dose-dependently inhibited OA-induced airway microvascular leakage and bronchoconstriction, and its effects were more potent in adrenalectomized animals (significant at 1 μ g/kg and higher) than in sham-operated animals (significant at 10 μ g/kg and higher). Although the plasma concentration of epinephrine during OA inhalation was approximately 3 times higher than that during saline inhalation in sham-operated animals, no difference was seen in adrenalectomized animals. In conclusion, while procaterol essentially possesses pronounced inhibitory effects on antigen-induced airway microvascular leakage and bronchoconstriction in guinea pigs, the effects are considerably masked by epinephrine released from the adrenal gland.

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Introduction

The guinea pig shows pulmonary responses and histological findings of the respiratory organ following antigen challenge that are similar or identical to those of asthmatic patients [1]. Furthermore, similar to the airway smooth muscle of human beings, the airway smooth muscle of guinea pigs responds well to many spasmogens, including histamine [2, 3], leukotrienes [3, 4], serotonin [5, 6], thromboxane A₂ [7, 8], and endothelins [9, 10]. The guinea pig has therefore been the preferred choice for use as a model of allergic bronchial asthma in the evaluation of anti-asthmatic drugs. However, it has been shown that antigen-induced bronchoconstriction in guinea pigs is attenuated by the action of epinephrine, which is released from the adrenal gland by an antigen-antibody reaction, on β_2 -adrenoceptors [11]. Epinephrine has been shown to exert antiexudative and bronchodilator effects by acting on α_1 -adrenoceptors and/or β_2 -adrenoceptors [12]. Therefore, when evaluating the effects of antiexudative and bronchodilative drugs in guinea pigs, there is the possibility that the effects of the drugs on antigen-induced airway responses may be influenced by adrenal epinephrine.

It is well known that selective β_2 -adrenoceptor agonists exhibit not only antiexudative effects but also bronchodilative effects in guinea pigs [13–16]. Procaterol is a potent and highly selective β_2 -adrenoceptor agonist [17–19]. Procaterol is known to be a full agonist at β_2 -adrenoceptors because the drug has been shown to have efficacy (intrinsic activity) similar to isoproterenol and epinephrine in relaxing isolated guinea-pig trachea precontracted maximally by carbachol [20]. Unlike partial agonists such as salbutamol and salmeterol, which have significantly less efficacy than full agonists [20], procaterol has no antagonistic action on β_2 -adrenoceptors. Procaterol also has neither agonistic [19] nor antagonistic [21] actions on α -adrenoceptors. Although there is little information on the effect of procaterol on antigen-induced airway microvascular leakage in guinea pigs, the drug has been shown to inhibit not only antigen-induced bronchoconstriction [22] but also airway microvascular leakage and bronchoconstriction induced by spasmogens such as histamine [15, 23] and eicosanoids [16, 23] in the animals. Taking this background into consideration, we used procaterol as a test drug for investigating the possible influence of the adrenal gland on the effects of antiexudative and bronchodilative drugs on antigen-induced airway responses in guinea pigs.

Thus, we examined the inhibitory effects of procaterol on antigen-induced airway microvascular leakage and bronchoconstriction in adrenalectomized guinea pigs and compared them with the effects in sham-operated animals. In addition, we also measured plasma catecholamine levels in order to assess their contribution to any difference in the inhibitory effects of procaterol on airway responses between adrenalectomized and sham-operated animals.

Materials and Methods

Preparation of Anti-Ovalbumin Guinea-Pig Serum

Four-week-old male Hartley guinea pigs were sensitized by intraperitoneal injection of 1 ml/animal of saline containing 5 μ g of ovalbumin (OA) and 20 mg of silica, a total of 4 times at 2-week intervals. Serum was collected from each animal 1 week after the last sensitization, pooled, and kept frozen until use. The 48-hour passive cutaneous anaphylaxis titer of the serum was 1:1,000.

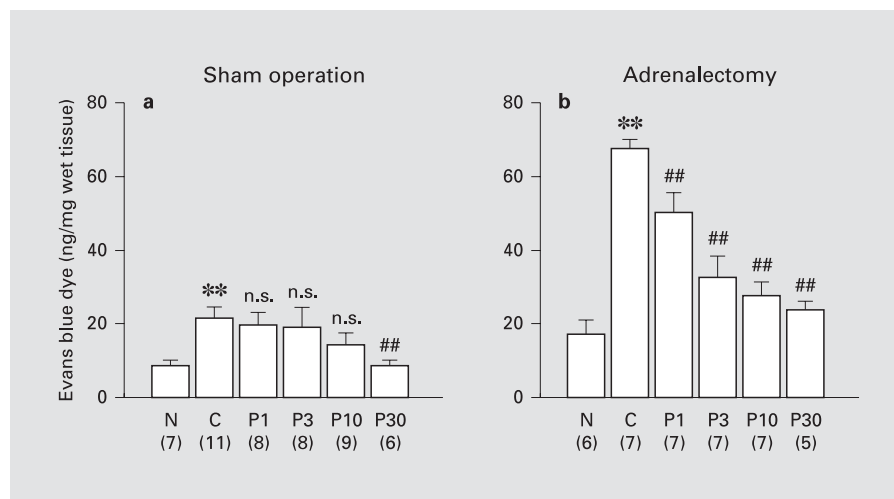
Animal Preparation and Measurement of Bronchoconstriction

At 7–8 weeks of age male Hartley guinea pigs were passively sensitized by intravenous injection of 0.5 ml/animal of anti-OA guinea-pig serum. Two days after passive sensitization, the guinea pigs were anesthetized with urethane (1.2–1.4 g/kg i.p.), a midline abdominal incision was made, and either a bilateral adrenalectomy or a sham operation was performed. 20 min after surgery, the guinea pigs were intranasally administered [24] procaterol dissolved in saline (0.9% NaCl) at doses of 1, 3, 10, or 30 μ g/kg at a volume of 0.3 ml/kg. Normal and control animals were administered saline at a volume of 0.3 ml/kg. 10 min after administration, a cannula was inserted into the trachea through a tracheostomy and Evans blue dye (20 mg/kg) dissolved in saline was injected into the right external jugular vein. The animals were then immediately connected to a rodent ventilator (Model 683, Harvard Apparatus) and ventilated with room air at a rate of 60 breaths/min and a tidal volume of 4 ml/animal. About 1 min later, OA (10 mg/ml in saline) or saline (for normal animals) was aerosolized using a nebulizer (Type A, Azwell) and delivered to the animals through the ventilator for 10 min while monitoring changes in pulmonary inflation pressure. Pulmonary inflation pressure was measured using a pressure transducer (Type 45363, NEC San-ei) connected to a side arm of the tracheal cannula and recorded on a recorder (Recti-Horiz 8K, NEC San-ei). Bronchoconstriction was determined as the change in pulmonary inflation pressure (Δ mm Hg) [11, 25]. The maximal change in pulmonary inflation pressure, usually obtained within 3 min after the start of OA inhalation, was analyzed.

Determination of Airway Microvascular Leakage

After the ventilator had been disconnected, the thoracic cavity was opened, a blunt 14-gauge needle was passed into the aorta from the left ventricle, and the ventricles were cross-clamped. Blood was expelled through an incision in the right atrium while perfusing the animal with about 200 ml of saline at a pressure of 90 mm Hg to remove intravascular dye from the systemic and pulmonary circulation until the perfusate became clear. The lungs were removed and the parenchyma was carefully scraped off using cotton swabs.

Fig. 1. Effects of procaterol on OA-induced extravasation of Evans blue dye into intrapulmonary airways in sham-operated (**a**) and adrenalectomized (**b**) guinea pigs. N = Normal animals; C = control animals; P1–P30 = animals pretreated with procaterol at doses of 1, 3, 10, and 30 $\mu\text{g/kg}$, respectively. Values are mean $\pm$ SEM; numbers in parentheses indicate number of animals. n.s. = Not significant. ** $p < 0.01$ vs. normal animals (Student's unpaired t test); ## $p < 0.01$ vs. control animals (Williams test).



The intrapulmonary airways were separated from the extrapulmonary airways (trachea and main bronchi) and weighed wet after blotting. The Evans blue dye was extracted with 2 ml of formamide in a 37°C water bath for 24 h and measured using a spectrophotometer (Viento, Bio-Tek Instruments) at 620 nm. The results were plotted against a standard curve for Evans blue dye (0–20 $\mu\text{g/ml}$) in formamide. The amount of Evans blue dye contained in samples was calculated and expressed as ng/mg tissue wet weight. Evans blue dye measurements have been shown to closely correlate with the extravasation of radiolabeled albumin in guinea pig airways [26].

Measurement of Plasma Catecholamine Levels

30 min after adrenalectomy or sham operation, OA or saline inhalation was performed as described above. A venous blood sample was collected from the large abdominal vein using a syringe 2–4 min after the start of the inhalation when the peak OA-induced increase in pulmonary inflation pressure was seen. The blood was immediately transferred to a sample tube containing EDTA-2Na and centrifuged at 3,000 rpm for 30 min, and the plasma was frozen for catecholamine analysis. Each sample comprised plasma obtained from 1 or 2 animals. Fractional catecholamine analyses (nor-epinephrine, epinephrine, and dopamine) were performed by HPLC electrochemical detection at the Department of Biomedical Research, SRL, Inc., Hachioji, Tokyo, Japan.

Drugs and Chemicals

The following drugs and chemicals were used: procaterol (Otsuka Pharmaceutical Co.), OA (Sigma, St. Louis, Mo., USA), silica (Sigma), urethane (ethyl carbamate, Sigma), Evans blue dye (Sigma-Aldrich, St. Louis, Mo., USA), formamide (Kanto Chemical Co., Tokyo, Japan), and physiological saline (Otsuka Pharmaceutical Co.).

Statistical Analysis

All data are expressed as the mean $\pm$ SEM. Overall, statistical differences were determined by Student's unpaired t test (two-

tailed) for comparison between 2 groups. The inhibitory effects of procaterol on airway microvascular leakage and bronchoconstriction were analyzed by regression analysis to determine the significance of dose-dependency. When the significance of dose-dependency was confirmed, Williams test (lower-tailed) was performed to assess the significance of differences between the control group and the procaterol groups. Statistical significance was based on a 95% confidence level. Statistical analysis was performed using the statistical package SAS (Statistical Analysis System, SAS Institute Japan, Release 8.1).

Results

Inhibitory Effect of Procaterol on Airway Microvascular Leakage (fig. 1)

In the normal groups, there was no significant difference in the amount of extravasated Evans blue dye between adrenalectomized and sham-operated animals (16.83 ± 4.07 vs. 8.87 ± 1.18 ng/mg tissue wet weight). Although significant and apparent increases were observed in both control groups in comparison with the corresponding normal group, the amount was markedly higher in adrenalectomized animals than in sham-operated animals (67.49 ± 2.75 vs. 21.72 ± 2.81 ng/mg tissue wet weight). While procaterol dose-dependently inhibited the dye extravasation in both groups, the inhibitory effect was much more potent in adrenalectomized animals than in sham-operated animals. Significant inhibition was observed even at the lowest dose of 1 $\mu\text{g/kg}$ in adrenalectomized animals, but only at the highest dose of 30 $\mu\text{g/kg}$ in sham-operated animals.

Fig. 2. Effects of procaterol on OA-induced increase in pulmonary inflation pressure in sham-operated (**a**) and adrenalectomized (**b**) guinea pigs. N = Normal animals; C = control animals; P1–P30 = animals pretreated with procaterol at doses of 1, 3, 10, and 30 $\mu\text{g}/\text{kg}$, respectively. Values are mean $\pm$ SEM; numbers in parentheses indicate number of animals. n.s. = Not significant. ** $p < 0.01$ vs. normal animals (Student's unpaired t test); ## $p < 0.01$ vs. control animals (Williams test).

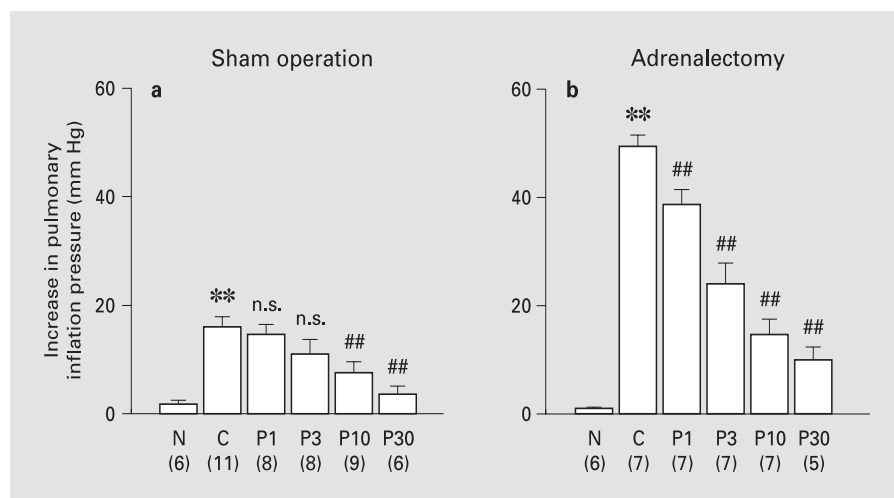


Table 1. Plasma catecholamine levels during saline or OA inhalation in sham-operated and adrenalectomized guinea pigs

Catecholamine	Treatment	Saline inhalation	OA inhalation
Norepinephrine pg/ml	Sham operation	3,809 $\pm$ 449 (6)	7,705 $\pm$ 1,462 (7) [#]
	Adrenalectomy	4,222 $\pm$ 897 (5)	8,814 $\pm$ 1,304 (6) [#]
Epinephrine pg/ml	Sham operation	22,943 $\pm$ 6,780 (6)	68,273 $\pm$ 14,350 (7) [#]
	Adrenalectomy	3,551 $\pm$ 287 (5)	4,424 $\pm$ 912 (6)
Dopamine pg/ml	Sham operation	342 $\pm$ 48 (6)	503 $\pm$ 78 (7)
	Adrenalectomy	409 $\pm$ 53 (5)	546 $\pm$ 36 (6)

Values are mean $\pm$ SEM; numbers in parentheses indicate number of samples. Each sample comprised plasma obtained from 1 or 2 animals. n.s. = Not significant.

* $p < 0.05$; ** $p < 0.01$ for comparison between sham-operated and adrenalectomized animals by Student's unpaired t test.

[#] $p < 0.05$ vs. saline inhalation by Student's unpaired t test.

Inhibitory Effect of Procaterol on Bronchoconstriction (fig. 2)

There was no significant difference in the change in pulmonary inflation pressure between the 2 normal groups. Significant and apparent increases in pulmonary inflation pressure were observed in both control groups in comparison with the corresponding normal group. Similar to the results for airway microvascular leakage, the increase in pulmonary inflation pressure was markedly greater in adrenalectomized animals than in sham-operated animals (50.00 ± 2.18 vs. 15.32 ± 2.28 mm Hg). Procaterol dose-dependently inhibited the increase in pulmonary inflation pressure in both groups, with the inhibitory effect being more potent in adrenalectomized animals (significant at 1 $\mu\text{g}/\text{kg}$ and higher) than in sham-

operated animals (significant at 10 and 30 $\mu\text{g}/\text{kg}$). The inhibitory effect of procaterol on bronchoconstriction paralleled its inhibitory effect on airway microvascular leakage.

Plasma Catecholamine Levels (table 1)

During saline inhalation, a significant difference in plasma epinephrine level, but not in norepinephrine or dopamine levels, was observed between adrenalectomized and sham-operated animals ($3,551 \pm 287$ vs. $22,943 \pm 6,780$ pg/ml). In both groups, the plasma norepinephrine level during OA inhalation was approximately twice as high as during saline inhalation. On the other hand, although the plasma epinephrine level during OA inhalation was approximately 3 times as high as dur-

ing saline inhalation in sham-operated animals, there was no difference in the plasma epinephrine level between OA inhalation and saline inhalation in adrenalectomized animals. There were no significant differences in plasma dopamine levels either between adrenalectomized and sham-operated animals or between OA inhalation and saline inhalation.

Discussion

In the present study, the airway responses to OA, i.e. airway microvascular leakage and bronchoconstriction, were markedly enhanced in adrenalectomized guinea pigs. Plasma concentrations of norepinephrine and epinephrine, but not dopamine, have been reported to increase when sensitized guinea pigs are exposed to an antigen [11]. The increased catecholamine levels are probably due to increases in sympathetic nerve discharges by the following mechanisms: direct stimulation of the sympathetic ganglia by histamine released from mast cells residing in the ganglia [27], and by means of vagal reflexes accompanying bronchoconstriction [28] evoked by inflammatory mediators released from mast cells. In the present study, plasma levels of norepinephrine, but not dopamine, were significantly higher in both adrenalectomized and sham-operated animals (approximately twice as high in both groups) during OA inhalation than during saline inhalation, indicating that sympathetic nerve discharges were increased to the same extent by the antigen in both groups. On the other hand, although an increase in plasma epinephrine level was observed in sham-operated guinea pigs (approximately 3 times as high) during OA inhalation, no such increase was observed in adrenalectomized guinea pigs. Taken together, these results suggest that the enhancement of OA-induced airway responses by adrenalectomy is due to the absence of adrenal epinephrine acting as a counter-regulatory hormone.

In the present study we clearly demonstrated that procaterol possesses pronounced inhibitory effects on antigen-induced airway microvascular leakage and bronchoconstriction in adrenalectomized guinea pigs. On the other hand, procaterol's inhibition of those airway responses in sham-operated animals was far less potent than in adrenalectomized animals. The reason for procaterol's weaker potency in sham-operated animals could be due to the participation of adrenal epinephrine. Epinephrine may possibly inhibit airway microvascular leakage not only by decreasing blood delivery to the leakage sites (i.e. postcapillary venules) due to α_1 -adrenoceptor-mediated

contraction of precapillary arterioles [12], but also by inhibiting gap formation between the endothelial cells of postcapillary venules due to β_2 -adrenoceptor-mediated relaxation of those endothelial cells [29, 30]. Epinephrine also induces β_2 -adrenoceptor-mediated relaxation of airway smooth muscle. Thus, if procaterol had an antagonistic action on α_1 -adrenoceptors and/or β_2 -adrenoceptors, it is likely that the drug's inhibitory potency would be weaker in sham-operated animals than in adrenalectomized animals due to attenuation of the inhibitory effects of adrenal epinephrine. However, procaterol has no antagonistic action on either β_2 -adrenoceptors [20] or α -adrenoceptors [21]. Therefore, a possible explanation for the weaker potency of procaterol in inhibiting antigen-induced airway responses in sham-operated animals than in adrenalectomized animals is simply as follows: the inhibitory effects of procaterol in sham-operated animals, i.e. in the presence of adrenal epinephrine, are masked by the inhibitory effects of adrenal epinephrine because of their mutual effective site, i.e. β_2 -adrenoceptors. Other antiexudative and bronchodilative drugs with a mechanism of action unrelated to β_2 -adrenoceptors, such as leukotriene receptor antagonists and histamine receptor antagonists [31], will need to be examined similarly.

In the present study, the inhibitory effect of procaterol on airway microvascular leakage paralleled its inhibitory effect on bronchoconstriction in both adrenalectomized and sham-operated guinea pigs. On the other hand, procaterol has been reported to have low efficacy on airway microvascular leakage when airway responses are induced by spasmogens such as histamine [15, 23] and eicosanoids [16, 23], i.e. inhaled procaterol only partially inhibited airway microvascular leakage even at doses that almost completely inhibited bronchoconstriction. In a preliminary study using non-adrenalectomized guinea pigs, we showed that intranasally administered procaterol at 100 $\mu\text{g/kg}$, a higher dose than those tested in the present study, inhibited exudation of Evans blue dye into the intrapulmonary airways and an increase in pulmonary inflation pressure induced by histamine, but dye exudation was inhibited only to about 50% of the control, while inhibition of the increase in pulmonary inflation pressure was almost complete (data not shown). The most likely explanation for the discrepancy between those previous findings and the results of the present study may be the difference in airway stimulation. There are two steps in the occurrence of the airway responses induced by antigen challenge. The first step is the release of inflammatory mediators, predominantly histamine and leukotrienes [31], from mast cells activated by the antigen [32–

34]. The second step is the direct contractile action of such inflammatory mediators on endothelial cells in postcapillary venules, which induces airway microvascular leakage by forming gaps between the endothelial cells [12], and on airway smooth muscles, which induces bronchoconstriction. Procaterol may inhibit the first step as well as the second step, since the drug has been shown to inhibit the release of chemical mediators from passively sensitized lung fragments [35] and also to inhibit spasmogen-induced airway microvascular leakage and bronchoconstriction [15, 16, 23] probably by inhibiting the formation of gaps between the endothelial cells lining the microvessels [29, 30] and by inhibiting airway smooth muscle contraction. Procaterol administered into the airways might be more easily accessible at high concentrations to mast cells distributed in the airway epithelium, which have been suggested to be responsible for initiation of the airway responses induced by inhaled antigen [32–34], than to endothelial cells in postcapillary venules [36]. Thus, the reason why procaterol shows more potent inhibitory efficacy for antigen-induced rather than spasmogen-induced airway microvascular leakage is thought to be the difference in the sites for the drug's inhibitory actions. While procaterol might inhibit both mast cells and endothelial cells in antigen-induced airway responses, its inhibition of mast cells may be predominant. On the other hand, endothelial cells, which do not have such easy access to high concentrations of procaterol administered into the airways, might be the predominant site of the drug's inhibitory action in spasmogen-induced airway responses. Further studies will be needed to support this hypothesis.

In conclusion, while procaterol essentially possesses pronounced inhibitory effects on antigen-induced airway microvascular leakage and bronchoconstriction in guinea pigs, the effects are considerably masked by epinephrine released from the adrenal gland. Furthermore, the results of the present study suggest that the participation of the adrenal gland should be taken into consideration when evaluating the effects of antiexudative and bronchodilative drugs on antigen-induced airway microvascular leakage and bronchoconstriction in guinea pigs.

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