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ET_A Receptor Blockade Potentiates the Bronchoconstrictor Response to ET-1 in the Guinea Pig Airway

James S. Polakowski,[1] Terry J. Opgenorth, and David M. Pollock[2]

Pharmaceutical Discovery, Abbott Laboratories, Abbott Park, Illinois 60064

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The effect of ET_A receptor blockade on the bronchopulmonary response to endothelin-1 was determined in the airway of the anesthetized, spontaneously breathing guinea pig. Endothelin-1 administered as an aerosol increased lung resistance and decreased dynamic lung compliance. Delivery of the ET_A receptor antagonist, FR139317, 5 min prior to giving endothelin-1 greatly potentiated these changes. A lower dose of endothelin-1 that had no effect on resistance or compliance produced large and significant changes when pretreated with FR139317. In contrast, aerosolized FR139317 had no effect on the bronchopulmonary response to intravenously administered endothelin-1. These data suggest a non-contractile function of ET_A receptors accessible from the airways that serve to buffer the constrictor effects of non-ET_A receptors.

Endothelin (ET-1) is a potent vasoconstrictor peptide that demonstrates significant bronchoconstrictor activity when administered intravenously or as an aerosol (1-5). At least two receptor subtypes are responsible for mediating the contractile properties of ET-1 in vascular tissue (6). ET_A and ET_B receptor subtypes are distinguished by the relative affinities of the various endothelin isoforms (7). ET-1 has a greater affinity for the ET_A receptor compared to ET-3 or sarafotoxin 6c. ET-1, ET-3, or sarafotoxin 6c have similar affinity for the ET_B receptor.

Both ET_A and ET_B receptor subtypes have been identified in the pulmonary vasculature and airway smooth muscle. In the pulmonary circulation of the guinea pig, ET_A receptors appear to mediate ET-1-induced contractions as demonstrated in vitro and in vivo (8-10). ET-1 can contract isolated airway tissues via both ET_A and ET_B receptor subtypes (8, 11-14). In the anesthetized, ventilated guinea pig, intravenous injection of ET-1 results in a potent pressor response along with an increase in airway resistance (15-16). Intravenous infusion of an ET_A receptor antagonist inhibited the pressor response to ET-1 but had little effect on bronchoconstriction suggesting that non- ET_A receptors are functional in bronchial smooth muscle (10). There have been no previous reports concerning the effect of ET_A receptor antagonists when administered via the airway.

The purpose of the current study was to further characterize the ET-1-induced bronchoconstrictor response in the anesthetized guinea pig when administered as an aerosol. More specifically, experiments were designed to investigate the effect of pre-treatment with the ET_A receptor antagonist, FR139317, on the bronchoconstrictor response to ET-1 when the antagonist is delivered through the airway. FR139317 is a highly selective ET_A receptor antagonist as demonstrated in a variety of in vitro and in vivo experiments (17-18).

[1] To whom correspondence should be addressed at Abbott Laboratories, D46R AP9, Abbott Park, IL 60064-3500. Fax: (1) 847–938–5286. E-mail: Jim.Polakowski@Abbott.com.
[2] Current address: Vascular Biology Center, Medical College of Georgia, Augusta, GA 30912–2500.

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MATERIALS AND METHODS

Male Hartley guinea pigs (400-600g, Charles River), were anesthetized with Inactin (90 mg/kg, i.p., synthesized in-house) and allowed to breathe spontaneously. The animals were placed on a thermostatically controlled heating pad to maintain core body temperature at 36-38°C. Endotracheal intubation was performed using a piece of PE240 tubing. PE50 catheters were inserted into the carotid artery and jugular vein and used for the measurement of arterial blood pressure and administration of compounds, respectively.

Airflow was measured by connecting the distal end of the endotracheal tube to a heated mesh screen pneumotachograph (Fleisch, Switzerland) which in turn was connected to a differential pressure transducer (Validyne, Northridge, CA). Intrapleural pressure was determined by the placement of a Millar ''Mikro tip'' catheter pressure transducer (Millar Instruments, Inc., Houston, TX) in the mid-esophagus. The air flow and intrapleural pressure signals were used to calculate lung resistance (R_L) and dynamic lung compliance (C_{dyn}) on a breath-by-breath basis by a MI^2 data acquisition system (Modular Instruments, Inc., Southeastern, PA) using the method of Amdur and Mead (19).

The guinea pigs were allowed to recover from surgery for 30 - 60 minutes to ensure stable readings. The MI^2 was scheduled to collect a 10 second data sample every minute, and once the animals were stable, 10 minutes of baseline data was collected. In all groups, FR139317 or vehicle was then administered 5 minutes before the ET-1 challenge. The 10 minutes of control data were averaged to give one value. The 5 minutes of treatment data (FR139317 or vehicle) were averaged to give one value. When ET-1 was given, data were averaged in 5 and 2 min intervals for aerosol and intravenous administrations, respectively.

Experiments were conducted on the following groups: 1) FR139317 at 30 mg/ml or vehicle (60 second aerosol) + ET-1 at 0.1 mg/ml (60 second aerosol), 2) FR139317 at 30 mg/ml or vehicle (60 second aerosol) + ET-1 at 0.01 mg/ml (60 second aerosol), and 3) FR139317 at 30 mg/ml or vehicle (60 second aerosol) + ET-1 at 0.25 μg/kg (i.v. bolus).

Aerosols were generated by a Devilbiss ''Ultra Neb 99'' ultrasonic nebulizer (Somerset, PA, USA) connected in series with the afferent limb of a small animal ventilator (Harvard Bioscience, South Natick, MA) as described by Lagente et al. (14). Aerosols were generated for 60 seconds at 60 breaths/minute delivering 1 ml air/100 g body weight directly into the trachea via the endotracheal tube.

ET-1 (American Peptide, Sunnyvale, CA, USA) was dissolved in distilled and deionized water at 1 mg/ml. For aerosol solutions, the stock solution was further diluted in 0.9% NaCl (saline). For intravenous administration, the stock solution was diluted in phosphate buffered saline containing 0.1% bovine serum albumin. FR139317 (synthesized in-house) was dissolved in 0.25N $NaHCO_3$ (pH $\sim$ 8.5).

The values are reported as mean $\pm$ SE. The difference between mean values of FR139317- and vehicle-treated guinea pigs were analyzed by Student's t test for unpaired data. Within each treatment, mean values were analyzed by analysis of variance (ANOVA) for repeated measures (Abacus Concepts, Statview II, Berkeley, CA, USA). Analysis was considered significant at $P < 0.05$. Animal protocols were approved by the Institutional Animal Care and Use Committee of Abbott Laboratories and were in full compliance with the National Institutes of Health guide.

RESULTS

An ET-1 aerosol challenge at 0.1 mg/ml for 60 s, resulted in a very small increase in R_L (29$\pm$5%), decrease in C_{dyn} (21$\pm$5%) with no change in MAP (Fig. 1). FR139317 administered as an aerosol (30 mg/ml for 60 s) had no effect on these variables during the 5 min prior to giving ET-1. However, FR139317 greatly potentiated ET-1-induced changes in R_L and C_{dyn} compared to vehicle treated animals (434$\pm$119% and 74$\pm$5%, respectively). R_L increased to a peak level in FR139317 treated animals that was significantly greater than vehicle treated animals ($P<0.004$). The decrease in C_{dyn} was significantly greater in the FR139317-treated group ($P<0.0001$). ET-1 had no effect on MAP in either group.

Pre-treatment with FR139317 An ET-1 aerosol challenge at a tenfold lower concentration (0.01 mg/ml for 60 s) also resulted in significant increases in R_L (19$\pm$2%) and decreases in C_{dyn} (14$\pm$3%) relative to baseline values (Fig. 2). Similar to the higher dose of ET-1, FR139317 pre-treatment (30 mg/ml for 60 s) resulted in a larger change in R_L (72$\pm$24%) and C_{dyn} (31$\pm$6%) in response to ET-1. Again, ET-1 had no effect on MAP in either group.

Aerosol pre-treatment with FR139317 (30 mg/ml for 60 s), 5 minutes before an i.v. bolus challenge of ET-1 (0.25 μg/kg) had no effect on ET-1-induced changes (Fig. 3). However, the time course of the ET-1 response was much shorter in duration compared to aerosol ET-1. MAP increased 20 $\pm$ 4% compared to 25 $\pm$ 2% in vehicle and FR139317-treated animals,

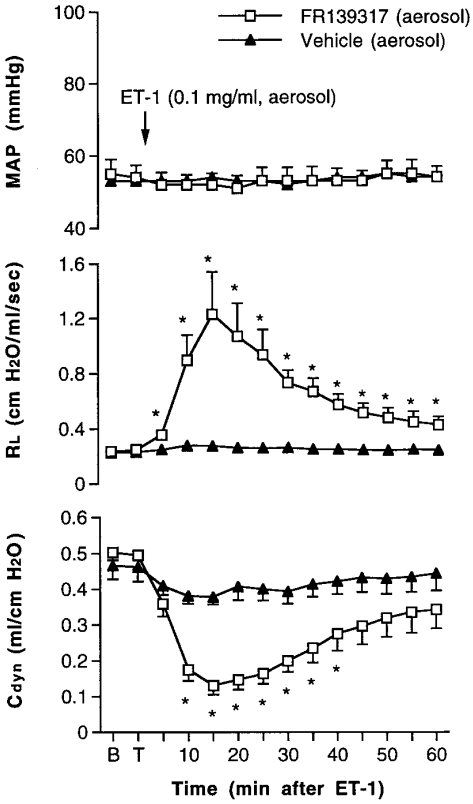

FIG. 1. Effect of ET-1 (0.1 mg/ml, 60 sec. aerosol) on MAP, R_L, and C_{dyn} in guinea pigs pretreated with a 60 sec aerosol of either FR139317 at 30 mg/ml (n=5) or vehicle (n=6) 5 min before the ET-1 challenge. B denotes values for the 10 min baseline period and T denotes values for the 5 min after pretreatment and before ET-1. * $P < 0.05$ vs. vehicle.

respectively. No significant differences were observed between vehicle and FR139317 treated animals.

DISCUSSION

Results from the present study provide new information about endothelin receptor subtypes and suggest a unique mechanism for ET_A receptors in the airways of the guinea pig lung. Local blockade of the ET_A receptor by aerosol administration of FR139317 potentiated the bronchoconstrictor response to a subsequent ET-1 challenge. These results suggest that ET_A receptor blockade unmasks the bronchoconstrictor activity of a non-ET_A receptor subtype consistent with in vitro and in vivo evidence that ET_B receptor agonists are potent bronchoconstrictors (10, 12, 20). The reason for why the response to ET_A receptor blockade was potentiated is not readily apparent and suggests a novel mechanism for the ET_A receptor in the guinea pig airway.

When ET-1 was administered through the airway, the bronchoconstrictor response was of much longer duration when compared to ET-1 given i.v. The temporal differences in the constrictor profile observed when comparing i.v. vs. airway delivery of ET-1 suggests different contractile mechanisms of ET-1 depending upon which side of the airway the peptide is administered. This idea is also supported by the fact that FR139317 potentiated the bronchocon-

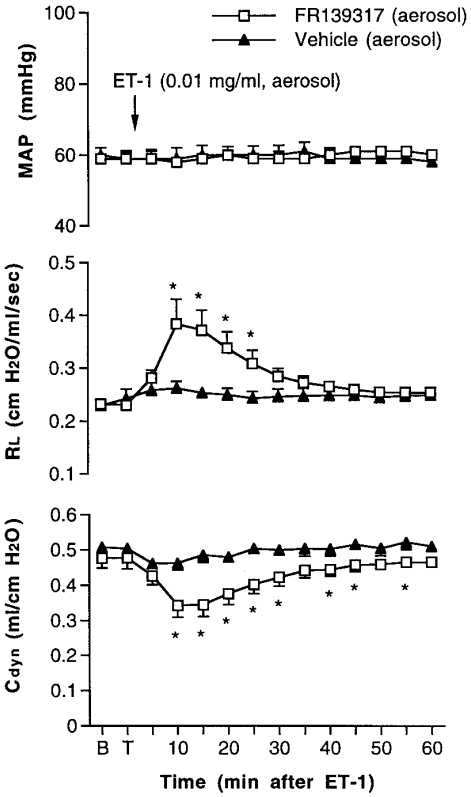

FIG. 2. Effect of ET-1 (0.01 mg/ml, 60 sec. aerosol) on MAP, R_L, and C_{dyn} in guinea pigs pretreated with a 60 sec aerosol of either FR139317 at 30 mg/ml (n=8) or vehicle (n=7) 5 min before the ET-1 challenge. B denotes values for the 10 min baseline period and T denotes values for the 5 min after pretreatment and before ET-1. * $P < 0.05$ vs. vehicle.

strictor response to aerosol but not i.v. ET-1. Inhaled ET-1 had no significant effects on blood pressure indicating that very little of the peptide reaches the circulation. Furthermore, these observations suggest that FR139317 does not readily pass through the alveoli or bronchi into the systemic circulation.

Using i.v. administration of the ET_A receptor antagonist BQ-123, Noguchi et al. observed inhibition of the bronchoconstrictor response to ET-1 given i.v. only when the antagonist was given at doses much higher than that required to block the systemic hemodynamic actions of ET-1 (10). Furthermore, Sorrentino et al. reported that i.v. BQ-123 had no effect on the bronchoconstrictor response to i.v. ET-1 while the non-selective antagonist, PD 145065, produced significant attenuation (21). The current study utilizing spontaneously breathing guinea pigs and a more potent and selective ET_A receptor antagonist, FR139317, confirms these findings in that ET-1-induced bronchoconstriction appears to be mediated by non-ET_A receptor activation.

Pharmacological evidence from in vitro studies indicate that ET_B receptors are located primarily within the airways and ET_A receptors in the parenchyma and vasculature (8, 20). Using autoradiography and isolated tracheal strips from the rat, Henry (14) reported that ET_A and ET_B receptors may coexist in tracheal smooth muscle. Inui et al. (22) have also localized both ET_A and ET_B receptors on single smooth muscle cells of the guinea pig trachea both of

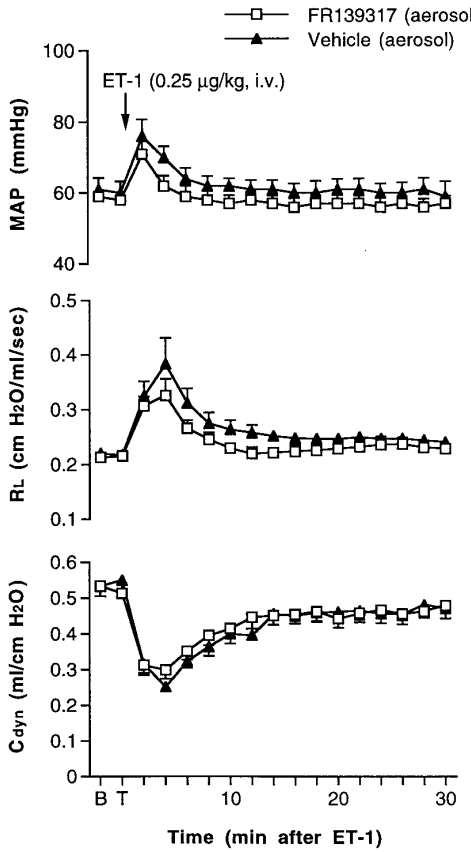

FIG. 3. Effect of ET-1 (0.25 μg/kg, i.v. bolus) on MAP, R_L, and C_{dyn} in guinea pigs pretreated with a 60 sec aerosol of either FR139317 at 30 mg/ml (n=7) or vehicle (n=5) 5 min before the ET-1 challenge. B denotes values for the 10 min baseline period and T denotes values for the 5 min after pretreatment and before ET-1.

which appear to be linked to a contractile mechanism. Nakamichi et al. has shown in porcine pulmonary tissues that blood vessels and bronchi are rich in ET_A and the lung parenchyma is rich in ET_B (23) consistent with the results of Abraham et al. in sheep (1). In contrast, Hay et al. have reported the opposite results in guinea pigs and thus are more in line with our own findings (9). Therefore, differences in receptor distribution may exist between species which has also been observed for other tissues such as the kidney (24-25), and these differences may contribute to the responses observed.

The observation that blockade of ET_A receptors by aerosol administration of FR139317 potentiated the bronchoconstrictor response to ET-1 given via the airway was unexpected relative to the many previous reports on the functional role of ET_A receptors. There are several possible explanations for our findings that will require further investigation. First, it is possible that ET_A receptors in the airway are unique in that they produce a relaxation that normally opposes ET_B-mediated constriction. This idea is supported by in vitro evidence from Battisini et al. suggesting that ET_A receptor activation in the tracheal epithelium stimulated release of prostanoids responsible for relaxation of tracheal smooth muscle (12). However, this explanation is contrary to the known role of ET_A and ET_B receptors in regulation of vascular tone (26). Another possibility is that airway ET_A receptors can

function simply as clearance receptors. Due to the potentiating effects of ET_A receptor blockade, it is possible that ET-1 has a higher affinity for the ET_A receptor compared to the non- ET_A receptor that mediates the bronchoconstriction. This possibility would be consistent with experiments in the rat kidney that provide evidence for a lower affinity subtype of the ET_B receptor, termed ET_{B2} (27). Indeed, Warner et al. have proposed further sub-typing of ET_B receptors as ET_{B1} mediating endothelium-dependent vasodilation and ET_{B2} mediating non-ET_A contractile effects (6). A third explanation for our results might be that heterogeneity in receptor localization exists along the airways such that ET_A receptors located in the upper airway will bind ET-1 as it enters so that ET_B receptors located in deeper regions are activated only when ET_A receptors are occupied. This hypothesis is not consistent with in vitro results that ET_B receptors are the primary subtype in the upper airways (9). In any event, our results indicate that ET_A receptors accessible from the airway are not linked to a constrictor mechanism.

In conclusion, our results in anesthetized, spontaneously breathing guinea pigs indicate that ET_A receptors accessible from the airways may act to relax bronchial smooth muscle or act as clearance receptors so that blockade of these receptors result in a marked potentiation of the bronchoconstrictor response, possibly mediated by an ET_B receptor.

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