Anti-inflammatory Actions of Enprofylline, a Modified Xanthine, in the Canine Forelimb

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It has been previously reported that enprofylline (3-propyl xanthine) prevents histamine-mediated edema formation in the guinea pig lung. To further assess the potential anti-inflammatory effects of enprofylline, we infused it intra-arterially into the canine forelimb before and during a local intra-arterial infusion of histamine (4 μg/min) while monitoring forelimb skin lymph parameters. Infusion of enprofylline at 2 mg/min significantly decreased forelimb arterial pressures and increased heart rate and pulse pressure. Subsequent infusion of histamine caused a further reduction in forelimb arterial pressures and an increase in lymph flow, protein concentration, and protein transport similar to that seen with the infusion of histamine alone. Infusion of enprofylline at 5 mg/min decreased forelimb arterial pressures and systemic pressure. Subsequent histamine infusion further reduced forelimb arterial pressures, but the increase in lymph parameters was markedly attenuated. Enprofylline infused at 10 mg/min also decreased forelimb arterial and systemic pressures, but subsequent histamine infusion was essentially without effect on lymph parameters. To assess the role of catecholamines in enprofylline-mediated attenuation of histamine edema formation, we infused enprofylline at 5 mg/min in the presence of a β2-receptor blockade produced by the intra-arterial infusion of ICI 118551. The effects of enprofylline and histamine on vascular pressures were similar to those seen in the absence of β2-receptor blockade, but lymph flow, protein concentration, and protein transport increased similar to that seen with histamine alone. These data indicate that enprofylline is capable of attenuating histamine-induced increases in microvascular permeability, but this action of enprofylline is of an indirect nature, mediated through the release of catecholamines. (Circulation Research 1989;64:235-242)

Bronchial smooth muscle contraction, mucus hypersecretion, and inflammatory bronchial edema are cardinal pathophysiologic manifestations of asthma.1 As early as the mid-19th century, a commonly prescribed therapy for asthmatics was strong coffee. Over the last decade, theophylline has become one of the most frequently prescribed oral drugs for asthmatics. The clinical benefits of xanthine treatment include bronchodilation, an increase in mucociliary transport, and prevention or attenuation of the bronchial microvascular permeability increase produced by such putative asthma mediators as histamine.2-4 The disadvantages of xanthine usage are the numerous side-effects, which include diuresis, central nervous system stimulation, and the potential for life threatening seizure, increased gastric acid secretion, increased intestinal motility, and diarrhea.5-6 In an attempt to retain the bronchodilator and anti-inflammatory characteristics of xanthines, while reducing its extrapulmonary deleterious effects, several modified xanthines have been developed. Enprofylline, which is 3-propyl xanthine, is such a compound. Persson and Kjellin7 have shown that intravenous administration of enprofylline prevents bronchial edema formation following histamine aerosol exposure in guinea pigs as measured by changes in the ratio of lung weight to body weight. To further characterize the anti-inflammatory potential of enprofylline, we have infused it intra-arterially in the canine forelimb at three dosages and during a local intra-arterial infusion of histamine. Alterations in microvascular permeability to macromolecules were assessed by measurement of forelimb skin lymph flow and protein concentration.

Several reports have suggested that xanthine administration results in an increased release of catecholamines.8-10 Since it is well known that catecholamines can block histamine-mediated edema formation11,12 through interaction with peripheral β2-receptors, we also infused histamine and enprofylline in the presence of a β2-receptor blockade produced by the local intra-arterial infusion of ICI 118551.
Materials and Methods

Adult mongrel dogs of either sex were anesthetized with sodium pentobarbital, intubated, and ventilated with room air. The forelimb nerves, the brachial artery, and the brachial and cephalic veins were isolated. The remaining skin and muscle of the forelimb were tightly compressed with tourniquets to limit collateral blood flow. Following the intravenous administration of sodium heparin, a prenodal lymph vessel was cannulated above the elbow with PE 10 tubing. This lymph vessel drains forelimb skin and paw. The forelimb was perfused at constant arterial inflow via the brachial artery with blood obtained from a cannulated femoral artery with the use of a pressure-independent pump. Pressures were measured from cannulas placed in a small dorsal skin vein and a small ventral skin artery of the paw, the aorta via the brachial artery, and the brachial artery via a cannulated side-branch. All pressures were recorded continuously and read every 10 minutes. Lymph samples were collected for 10-minute periods in miniature graduated cylinders fashioned from plastic serological pipets. Arterial blood samples were obtained from a cannulated femoral artery for the determination of arterial plasma total protein concentration. Total protein concentrations were measured by the spectrophotometric method of Waddell. Lymph total protein transport was calculated by multiplying lymph flow in milliliters per 10 minutes times lymph total protein concentration in milligrams per milliliter to yield total protein transport in milligrams per 10 minutes.

The experimental protocol was as follows: lymph was collected and pressures were measured for a minimum of three 10-minute periods to ensure that a steady state had been reached. In the experiments involving histamine infusion alone, histamine (4 μg/min) was infused into the forelimb perfusion circuit behind the blood pump for a total of 60 minutes. In the histamine and enprofylline experiments, after the acquisition of control values, enprofylline (2, 5, or 10 mg/min) was infused into the forelimb perfusion circuit for a total of 80 minutes. Twenty minutes into the enprofylline infusion, an infusion of histamine (4 μg/min) was begun and continued for a total of 60 minutes. In the experiments involving β2-receptor blockade, after acquisition of control values, a bolus injection of 500 ng terbutaline in 1 ml NaCl was made into the forelimb perfusion circuit to establish a control response for β2-receptor stimulation. When vascular pressures had returned to control values, an infusion of the β2-receptor antagonist ICI 118551 (25 μg/min) was begun into the forelimb and continued for 100 minutes. Ten minutes into the ICI 118551 infusion, the injection of terbutaline was repeated to assess the effectiveness of the β2-receptor blockade. Twenty minutes into the ICI 118551 infusion, an infusion of enprofylline (5 mg/min) was begun and continued for 80 minutes. Twenty minutes later, an infusion of histamine (4 μg/min) was begun and continued for 60 minutes. In a final series of six experiments, the enprofylline vehicle (NaOH) was infused for a total of 80 minutes. In all experiments, arterial blood samples were collected immediately before the histamine infusion and 30 and 60 minutes thereafter for the measurement of plasma total protein concentrations.

Histamine diphosphate (Sigma Chemical, St. Louis, Missouri) and terbutaline (Draco, AB, Lund, Sweden) were suspended in normal saline. Enprofylline (Draco, AB) was dissolved in aqueous sodium hydroxide (55 mg/ml, pH 10.1). The identical sodium hydroxide solution was used in the vehicle control experiments. ICI 118551 (Stuart Pharmaceuticals, Wilmington, Delaware) was suspended in distilled water, heated to 37° C, and then salted with sodium chloride to bring the osmolarity up to 290 mosm. All solutions were made fresh daily and infused at a volume flow rate of 0.4 ml/min with an infusion pump (Harvard Apparatus, South Natick, Massachusetts), with the exception of terbutaline, which was injected. All data were analyzed with a factorial analysis of variance with differences between the means of the treatments determined by the method of Duncan.

Results

The intra-arterial infusion of histamine significantly decreased forelimb perfusion and skin small artery pressures (Figure 1). Systemic pressure, pulse pressure, heart rate, and forelimb skin small vein pressure were not significantly altered. Skin lymph flow and lymph total protein transport were significantly increased by minute 10 of the infusion period, whereas lymph total protein concentration was significantly increased by minute 30 (Figure 2).
Plasma total protein concentration was not changed by histamine infusion (Table 1).

The local intra-arterial infusion of enprofylline at 2 mg/min for 20 minutes significantly decreased forelimb perfusion and skin small artery pressures without significantly affecting systemic pressure or skin small vein pressure (Figure 3). Enprofylline infusion did not change lymph flow, protein concentration, or protein transport (Figure 4). Heart rate was significantly increased as a result of enprofylline administration. The subsequent infusion of histamine during the continued infusion of enprofylline resulted in further decreases in forelimb perfusion and skin small artery pressures but no change in skin small vein pressure (Figure 3). Systemic pressure was decreased from minute 40 onward. This decrease is attributable to the continued infusion of enprofylline since systemic pressure is not significantly altered by histamine infusion alone. Pulse pressure was transiently increased (Table 1). Lymph flow and lymph total protein transport were significantly elevated by minute 10 whereas lymph protein concentration was elevated by minute 20 (Figure 4). Plasma total protein concentration was not affected (Table 1).

Infusion of enprofylline at 5 mg/min decreased forelimb perfusion, skin small artery, and systemic pressures without changing small vein pressure (Figure 5). Heart rate and pulse pressure were significantly increased by enprofylline (Table 1). Lymph parameters were not affected by the infusion of enprofylline (Figure 6). Subsequent infusion of histamine resulted in an additional decrease in forelimb perfusion and skin small artery pressures and a slight but significant decrease in skin small vein pressure (Figure 5). Lymph flow and total protein transport were significantly increased by minute 20 of the histamine infusion period and lymph protein concentration was significantly increased by minute 30 (Figure 6), but the increases were attenuated compared with those seen with the infusion of histamine alone (Figure 2).

Enprofylline infused at 10 mg/min significantly reduced forelimb perfusion, small artery, and systemic pressures, did not affect small vein pressure (Figure 7), but increased heart rate and pulse pressure (Table 1). Lymph parameters were not changed (Figure 8). Infusion of histamine during the continued infusion of enprofylline further reduced forelimb arterial pressures and caused a decrease in skin small vein pressure (Figure 7). Lymph flow was significantly increased only at minute 30 of the histamine infusion period, whereas lymph total protein concentration and total protein transport were not significantly altered (Figure 8).

In experiments in which a β-receptor antagonist was used, injection of 500 ng of the β-agonist terbutaline in 1 ml of saline reduced forelimb perfusion pressure from a control value of 130 mm Hg to a nadir of 96 mm Hg. A 1-ml bolus injection of saline decreased perfusion pressure from a control of 128 mm Hg to 125 mm Hg. When the injection of terbutaline was repeated 10 minutes into the ICI 118551 infusion, perfusion pressure was decreased from 136 mm Hg to a nadir of 132 mm Hg, whereas injection of 1 ml saline decreased perfusion pressure from 137 mm Hg to 134 mm Hg. These data indicate that an effective β-receptor blockade had been attained with ICI 118551 infusion.
Infusion of ICI 118551 intra-arterially at 25 μg/min did not affect systemic or forelimb pressures (Figure 9), heart rate or pulse pressure (Table 1), or lymph parameters (Figure 10). Subsequent infusion of enprofylline at 5 mg/min during the continued infusion of ICI 118551 significantly decreased forelimb perfusion, skin small artery, and systemic pressures without affecting small vein pressure (Figure 9) or lymph parameters (Figure 10). Infusion of histamine resulted in a further decrease in forelimb perfusion and skin small artery pressures and a slight but significant decrease in small vein pressure (Figure 9). Lymph flow was significantly increased by 10 minutes of the histamine infusion period while lymph protein concentration and protein transport were significantly increased by minute 20. The increases in lymph parameters in these experiments were similar to those seen during the infusion of histamine alone (Figure 2).

Infusion of the enprofylline diluent (NaOH, pH 10.1) did not affect vascular pressures or lymph parameters. Subsequent infusion of histamine significantly decreased forelimb perfusion and skin small artery pressures from minute 10 of the histamine infusion period onward, whereas skin small vein and systemic pressures were not significantly altered. Lymph flow, protein concentration, and protein transport were significantly increased from minute 10 onward. The changes in vascular pressures and lymph parameters observed when histamine was infused during the simultaneous infusion of the enprofylline diluent were very similar to those seen during the infusion of histamine alone.

### Discussion

Local intra-arterial infusion of histamine in the canine forelimb significantly increases microvascular permeability to macromolecules as evidenced by increases in skin lymph flow, protein concentration, and protein transport. Intravital microscopy has revealed that this increase in vascular permeability occurs at the level of the postcapillary venule. It has been proposed that histamine causes contraction of contractile filaments within the vascular endothelial cell with the result that the cells become rounded and the intercellular junctions separate. If, indeed, this is the mechanism by which histamine and other putative inflammatory mediators increase vascular permeability, then agents that relax contractile filaments could be expected to attenuate the permeability increase produced by inflammatory mediators. It is known that the local application of catecholamines can block histamine and bradykinin-mediated edema formation and that this action is mediated through peripheral β-receptor stimulation. Likewise, local administration of the β-receptor agonist terbutaline also prevents the increase in microvascular permeability to subsequently infused histamine or bradykinin.

It would be predicted, therefore, that xanthines in vaso dilatory or bronchodilator doses might also possess anti-inflammatory capabilities. Persson et al have shown that the increase in guinea pig lung weight that is seen after exposure to aerosol histamine can be blocked by the prior treatment of the animal with theophylline.
Enprofylline and Edema Formation

The clinical drawback to the use of theophylline in such conditions as chronic asthma, static asthmaticus, and adult respiratory distress syndrome is the numerous undesirable and even potentially fatal side-effects of theophylline. Much of the side-effects of theophylline can be attributed to its efficacy as an adenosine-receptor antagonist. Enprofylline, however, is without significant adenosine-antagonistic properties.

In the present study, the local intra-arterial infusion of histamine at 4 μg base/min significantly increased forelimb lymph flow, lymph protein concentration, and protein transport. Pretreatment of the forelimb by the local intra-arterial infusion of enprofylline at 2 mg/min does not significantly affect the ability of subsequently infused histamine to increase lymph parameters. However, infusion of enprofylline at 5 mg/min does significantly attenuate (p<0.05, group T) the effects of subsequent histamine infusion on lymph flow, protein concentration, and protein transport. The infusion of enprofylline at 10 mg/min essentially blocks the lymphatic effects of histamine. These data indicate that the local admin-
istration of enprofylline is capable of thwarting the action of histamine to increase microvascular permeability to plasma proteins.

These data are in agreement with results reported by Persson et al in guinea pig lung. Likewise, Thomas and Duarte have shown that the intravenous administration of enprofylline inhibited the increased plasma protein leakage produced by the intradermal injection of histamine and a number of putative inflammatory mediators in the rat.

The mechanism of action of the xanthines remains open to question. Certainly, in very large doses, xanthines are capable of inhibiting phosphodiesterase. However, in clinically applicable dosages, little if any phosphodiesterase inhibition is seen. It is also well known that theophylline, in clinically applicable dosages, displays a potent adenosine-receptor antagonism. However, enprofylline does not appear to possess any appreciable adenosine-antagonistic properties.

It has been suggested that part of the actions of xanthines may be mediated through increased circulating levels of catecholamines. Peach has reported that theophylline is capable of increasing the release of catecholamines from isolated cat adrenal glands. Snider and Waldock reported that theophylline administered intraperitoneally in rats significantly increased the synthesis of adrenal catecholamines. Robertson et al reported that a single oral dose of caffeine in man resulted in a 75% increase in plasma norepinephrine levels and a 207% increase in plasma epinephrine levels. Esquivel et al have reported that infusion of enprofylline in human subjects results in a 52% increase in circulating plasma epinephrine levels and a 47% increase in plasma norepinephrine levels. In the present study, all three dosages of enprofylline resulted in a significant decrease in systemic pressure. With the lowest dose of enprofylline, this decrease did not become significantly less than control until minute 40 of the histamine infusion period, whereas with the two higher dosages, systemic pressure was significantly decreased before the histamine infusion was begun. The decrease seen with the lowest infusion rate of enprofylline is clearly the result of the xanthine since animals receiving histamine alone or the enprofylline vehicle and histamine did not manifest any changes in systemic pressure. The
diminution of systemic pressure after the intra-arterial administration of vasodilatory dosages of xanthines has been previously reported. Thus, in addition to any direct release of adrenal catecholamines produced by enprofylline, a sympathoadrenal discharge via the baroreceptor reflex due to enprofylline’s potent vasodilator actions would be expected. Since it is known that the permeability increasing effects of histamine can be blocked by the local administration of catecholamines, the ability of enprofylline to block histamine edema formation could be of an indirect nature and mediated through catecholamines. Indeed, in the current study, when the infusions of enprofylline (5 mg/min) and histamine were repeated in the presence of a β2-receptor blockade produced by ICI 118551, no inhibition of the actions of histamine on microvascular permeability were observed.

These data suggest that the anti-inflammatory actions of enprofylline in the canine forelimb are mediated through increased circulating levels of catecholamines. Clearly, enprofylline itself is not interacting with peripheral β2-receptors since it produces as much or more vasodilation in the presence of a β2-receptor blockade than without β2-receptor blockade.

The current results indicating that enprofylline’s anti-inflammatory actions are likely mediated through catecholamine-induced stimulation of peripheral β2-receptors are in agreement with some previous studies. Persson et al2 reported that the intraperitoneal injection of propranolol did not significantly affect the ability of theophylline to block the increase in lung weight seen in guinea pigs after histamine aerosol exposure. Likewise, Thomas and Duarte4 reported that pretreatment of rats with propranolol did not affect the ability of enprofylline to attenuate the plasma protein leakage produced by the intradermal injection of histamine. They did however, report that the anti-inflammatory actions of enprofylline were significantly diminished in adrenalectomized animals. In neither of these studies was any test of the efficacy of the β2-receptor blockade reported. In the current study, an effective β2-receptor blockade was established by the infusion of ICI 118551 as evidenced by the total abolition of the response to injected terbutaline. In the presence of this blockade, enprofylline did not significantly affect histamine-mediated increases in lymph flow, protein concentration, and protein transport. In support of the current study, Svensjo and Roempke22 have reported that the anti-inflammatory effects of intravenous enprofylline and theophylline are blocked by the administration of ICI 118551 in the hamster cheek pouch.

Enprofylline has several properties that make it a potentially attractive alternative to theophylline. Enprofylline has been shown to be approximately five times more potent as a bronchodilator than theophylline. Like theophylline, enprofylline also increases mucociliary transport. However, enprofylline has been shown to lack many of the deleterious side-effects seen with theophylline administration.21 Additionally, enprofylline is excreted intact (80–100% of an oral dose), whereas less than 15% of an oral dose of theophylline is eliminated unchanged in the urine.26 Thus, maintenance of effective blood levels is much easier with enprofylline than theophylline.27

In summary, the local intra-arterial infusion of enprofylline is capable of significantly attenuating histamine-mediated edema formation in the canine forelimb. This action of enprofylline, however, is dependent upon an interaction with the peripheral β2-receptors and is thus mediated by way of either a direct and/or reflex increase of circulating catecholamines.

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