Dose-response relationship between plasma epinephrine concentration and alveolar liquid clearance in dogs

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Maron, Michael B. Dose-response relationship between plasma epinephrine concentration and alveolar liquid clearance in dogs. J. Appl. Physiol. 85(5): 1702–1707, 1998.—Previously, alveolar liquid clearance (ALC) was observed to increase in a canine model of neurogenic pulmonary edema (NPE) by adrenal epinephrine (S. M. Lane, K. C. Maender, N. E. Awender, and M. B. Maron. Am. J. Respir. Crit. Care Med. 158: 760–768, 1998). In this study the dose-response relationship between plasma epinephrine concentration and ALC was determined in anesthetized dogs by infusing epinephrine to produce plasma concentrations of 256 ± 37, 1,387 ± 51, 15,737 ± 2,161, and 363,997 ± 66,984 (SE) pg/ml (n = 6 for each concentration) for 4 h and measuring the resultant ALC. The latter was determined by mass balance after instillation of autologous plasma into a lower lung lobe. These plasma concentrations produced ALCs of 14.3 ± 1.2, 20.5 ± 1.9, 30.1 ± 1.5, and 37.9 ± 2.7% of the instilled volume, respectively. ALC after the lowest infusion rate was not different from that previously observed under baseline conditions (14.1 ± 2.1%), whereas in a previous study of NPE, plasma epinephrine concentration increased to 7,683 ± 687 pg/ml and ALC was 30.4 ± 1.6%. These data indicate that, during recovery from canine NPE, ALC is not maximally stimulated and suggest that it might be possible to pharmacologically produce further increases in the rate of resolution of this form of edema.

pulmonary edema; lung fluid balance; alveolar epithelium; β-adrenergic agonist; cardiac dysfunction

The results of studies conducted during the last decade and a half have indicated that the lung may play an active role in clearing edema fluid from its air spaces through the active transport of sodium (38). Specifically, sodium is thought to enter the alveolar epithelial type II cell through multiple specialized pathways located in the apical membrane and then to be pumped out of the basolateral side by the enzyme Na⁺-K⁺-ATPase. The resulting transepithelial osmotic pressure gradient allows water to be passively reabsorbed, and recent work suggests that the alveolar epithelial type I cell may provide an important pathway for the removal of water from the air spaces (9). The rate of sodium and, consequently, water transport has been shown to be increased by the administration of β₂-adrenergic agonists, such as terbutaline and epinephrine (2, 3, 7, 8, 10, 13, 14, 16, 35, 36, 39), and recent studies have provided evidence that endogenous epinephrine may increase the rate at which fluid is cleared from the air spaces during recovery from pulmonary edema produced by neurological insults (18) and sepsis (33) and may protect against and/or accelerate recovery of edema resulting from hemorrhagic shock (25, 32).

Previously, in a canine model of neurogenic pulmonary edema (NPE), the edema appeared to resolve rapidly (22). This form of edema may occur in individuals with head injuries, subarachnoid hemorrhage, seizures, or other stimuli that produce a centrally mediated massive activation of the sympathetic nervous system (SNS) (23). Recently, it was reported that epinephrine released from the adrenal glands was responsible for accelerating the rate at which excess fluid was absorbed from the air spaces during the recovery phase in this model (18). An unanswered question, however, is the degree to which alveolar liquid clearance (ALC) is stimulated by endogenous epinephrine during recovery from this form of edema in relation to the maximal capacity for epithelial β₂-adrenergic stimulation. This issue may have potential clinical significance in view of recent speculation that it might be possible to increase the rate of edema resolution in patients with pulmonary edema by administering β₂-adrenergic agonists (1). The efficacy of such therapy, however, would rely on the degree to which ALC might already be upregulated by endogenous β₂-adrenergic agonists. Specifically, if the naturally occurring increase in ALC is the maximal possible, the administration of additional β₂-agonists would not be effective. To answer this question, in this study the dose-response relationship between plasma epinephrine concentration and ALC was determined in anesthetized dogs by infusing epinephrine to produce a range of steady-state plasma epinephrine concentrations. The dose-response relationship was then used to determine whether the increase in ALC that was previously observed during the recovery phase of NPE (18) represented a state of maximal stimulation.

During the development of NPE, extreme increases in plasma epinephrine concentration develop (19). It is not clear, however, whether such high values are required to produce an increase in alveolar epithelial sodium and water transport or whether ALC can be stimulated by lower plasma concentrations. Examination of the lower range of the dose-response relationship would thus allow one to determine whether ALC is stimulated under conditions in which the degree of sympathetic activation is not as extreme. In this regard, I was particularly interested in the possibility that increases in plasma epinephrine concentration of a magnitude that occurs during intense exercise might increase ALC.
METHODS

Experiments were performed on 24 dogs of mixed breed and gender (21.4 ± 4.8 [SD] kg). The animals were anesthetized with pentobarbital sodium (30 mg/kg iv), intubated, and ventilated with a piston respirator. Catheters were placed in the right femoral and pulmonary (Swan-Ganz thermodilution catheter) artery to monitor arterial (Pa), pulmonary arterial (Ppa), and wedge (Pw) pressures and in the right femoral vein for intravenous infusions. Additional large-bore catheters were placed in the right carotid artery and jugular vein to be used as described below. The animals were ventilated with 30% O2 at an average frequency of 8.7 ± 1.2 breaths/min and a tidal volume of 452 ± 83 ml. The average end-inspiratory pressure was 9.0 ± 1.1 Torr. Arterial blood gases were analyzed using a Radiometer system and were as follows under baseline conditions: PO2 139.8 ± 10.1 Torr, PCO2 36.5 ± 2.8 Torr, and pH 7.40 (range 7.35–7.46). A polyethylene catheter (3 mm ID) was placed in a lower lung lobe airway through a port in the endotracheal tube to allow plasma to be instilled in the air spaces. Body temperature was measured from the thermistor on the Swan-Ganz catheter and was maintained by a water-perfused heating pad placed beneath the animal.

Determination of ALC. ALC was determined using the method of Berthiaume et al. (2, 3). For each experiment, ~120 ml of arterial blood were drawn from the animal, centrifuged, and replaced with an equal volume of 6% dextran. Plasma (3 ml/kg) was instilled in a lower lung lobe, and the increase in the instillate protein concentration that occurred as fluid was absorbed from the air spaces was used to calculate ALC by mass balance, as described below. The volume of instilled plasma remaining in the lung lobe at the end of the experiment (Vf) was calculated as (2, 3)

\[ V_f = V_i (C_i/C_f) \]  

(1)

where \( V_i \) is the initial instilled volume, \( C_i \) is the initial instillate protein concentration, and \( C_f \) is the final protein concentration. ALC, expressed as a percentage of the volume instilled, was calculated as (37)

\[ ALC = \frac{[V_f (F_{w,i}) - (V_i F_{w,i})]100}{V_f F_{w,i}} \]  

(2)

where \( F_{w,i} \) and \( F_{w,f} \) are the water fractions of the plasma instillate at the beginning and end of the experiment, respectively. These were determined gravimetrically.

The protein concentration of the instilled plasma was determined by refractometry (American Optical, Buffalo, NY). The American Optical instrument has been shown to accurately measure the increase in alveolar instillate protein concentration that occurs as fluid is absorbed from the air spaces under baseline conditions and when ALC was increased by terbutaline administration or massive SNS activation (14).

Experimental protocol. After the preparation had stabilized, baseline vascular pressures and thermodilution cardiac outputs (American Edwards Laboratories, Santa Ana, CA) were measured, and a 1-ml arterial blood sample was drawn for the determination of blood gases, hematocrit (microhematocrit), and plasma protein concentration. An additional 5-ml arterial sample was drawn for the analysis of plasma catecholamine concentrations by HPLC, as previously described (19). The plasma was then instilled in the lung, and additional hemodynamic measurements were made after 5 min. After completion of these measurements, an intravenous epinephrine infusion (see below) was started. Subsequent hemodynamic measurements were made 10 min after the epinephrine infusion was started, at 30 min, and then again at 30-min intervals for 4 h. Blood gases were determined at periodic intervals and maintained by administering bicarbonate and adjusting the ventilator. Hematocrit and plasma protein concentrations were determined at hourly intervals. Additional plasma catecholamine determinations were made at 10 min and again at 1, 2, and 4 h after the infusion was started. At the end of 4 h the animal was euthanized with an overdose of pentobarbital sodium and the lungs were removed. At this time a well-mixed alveolar fluid sample was collected for protein analysis to be compared with similar measurements made on the initial instillate.

Four groups of animals (n = 6 each) were studied, with the animals of each group receiving one of the following epinephrine (Sigma Chemical, St. Louis, MO) infusion rates: 9.3, 88.2, 923.3, and 9,274.0 ng·kg\(^{-1}\)·min\(^{-1}\). These infusion rates were selected to provide a range of plasma epinephrine concentrations below and above that observed after massive SNS activation (18). The infusion rates were selected using the data of Clutter et al. (6), who observed a linear relationship between epinephrine infusion rate and steady-state plasma epinephrine concentration in humans and that, at a given infusion rate, plasma epinephrine concentration remained constant once steady-state values had been achieved.

The two higher infusion rates caused Ppa to increase. To prevent pulmonary vascular pressure from reaching values that could produce edema (which would confound the mass balance determination of ALC), the carotid arterial catheter was opened in these studies as soon as Ppa started to rise, thus allowing blood to empty into a stirred, heated, heparinized reservoir. The volume of blood removed from the animals in this manner averaged 383 ± 98 and 517 ± 137 (SD) ml for the 923.3 and 9,274 ng·kg\(^{-1}\)·min\(^{-1}\) infusion groups, respectively. Between 30 and 60 min after the epinephrine infusion was started, it was possible to return the exsanguinated blood to the jugular vein using a roller pump without increasing Ppa. This procedure was previously found to prevent any edema formation after massive SNS activation or epinephrine administration (18).

Data analysis. Data from the four epinephrine infusion groups were compared with measurements made in a control group of animals (n = 6) from a previous study in which ALC was determined under baseline conditions (18). The latter animals (n = 6) were instrumented identically, and all measurements were made at the same time points as in this study. Differences in ALC, plasma norepinephrine concentrations, and hemodynamics between groups were evaluated by ANOVA. For the ALC and norepinephrine analyses the ANOVAs were followed by a Student-Newman-Keuls test to determine individual differences. For the cardiac output and hematocrit data, whether there were linear or curvilinear relationships between epinephrine dose and the average value for these variables between 10 and 240 min of epinephrine infusion were specifically tested. Paired comparisons were made using a paired Student's t-test.

RESULTS

Plasma catecholamine concentrations. The average plasma epinephrine concentrations for the four infusion rates of 9.3, 88.2, 923.3, and 9,274.0 ng·kg\(^{-1}\)·min\(^{-1}\) were 256 ± 37, 1,367 ± 51, 15,737 ± 2,161, and 363,997 ± 66,984 (SE) pg/ml, respectively (Fig. 1). The equivalent molar plasma concentrations were 1.4 × 10\(^{-9}\), 7.6 × 10\(^{-9}\), 8.6 × 10\(^{-8}\), and 2.0 × 10\(^{-6}\) M, respectively. The average plasma epinephrine concentration for the control group from a previous study was
Plasma norepinephrine concentration increased slightly (from 123 ± 20 to 181 ± 28 pg/ml, P < 0.05) during the experiment in the groups receiving the three lowest epinephrine infusion rates. A similar upward drift in plasma norepinephrine concentration was previously observed (18) in animals studied under baseline conditions. In contrast, in the highest epinephrine infusion group, norepinephrine increased from a mean baseline value of 195 ± 30 to 301 ± 47 pg/ml (P < 0.05) by 10 min after the infusion was started. A further increase (P < 0.05) was observed at 1 h (746 ± 142 pg/ml) and was maintained for the duration of the experiment (661 ± 111 and 675 ± 154 pg/ml at 2 and 4 h, respectively). Although the reason for this increase is not clear, previously it was found that higher plasma norepinephrine concentrations (up to 3,000 pg/ml) do not increase ALC (18).

Hemodynamic changes. Plasma instillation into the air spaces produced small but significant (P < 0.001) increases in Ppa [3.6 ± 2.6 (SE) Torr] and Pa (2.7 ± 0.7 Torr) but no significant change in Pw (Figs. 2 and 3). Ppa and Pw were maintained at low levels throughout the experiment in all groups (Fig. 2). Pa remained relatively constant in the groups receiving the three lowest epinephrine infusion rates (9.3, 88.2, and 923.3 ng·kg⁻¹·min⁻¹) but increased substantially soon after the highest (9,274 ng·kg⁻¹·min⁻¹) infusion was started (Fig. 3). After this time, Pa fell progressively over the course of the experiment to levels below baseline. Cardiac output increased during the three lowest infusions and remained elevated throughout the experiment (Fig. 4). The highest infusion, however, caused cardiac output to progressively fall over the 4-h observation period. Analysis of the relationship between epinephrine dose and the average cardiac output between 10 and 240 min of epinephrine infusion further indicated a significant (P < 0.0001) quadratic relationship between these variables (Fig. 5). In other words, the two lowest doses of epinephrine increased cardiac output in a dose-dependent fashion, the 923.3 ng·kg⁻¹·min⁻¹ infusion rate resulted in a smaller average increase, and the highest dose caused cardiac output to actually fall below baseline values. Epinephrine infusion significantly (P < 0.0001) increased hematocrit in a dose-dependent manner (Fig. 6). Although a slight increase in hematocrit was observed during the lowest epinephrine infusion, hematocrit increased to a similar degree in the control group. No changes in plasma...
protein concentration occurred in any of the groups (data not shown).

ALC. In the control group from a previous study (18), the alveolar fluid protein concentration increased 15.3 ± 2.6% over 4 h under baseline conditions. In the four epinephrine infusion groups, alveolar fluid protein concentration increased (from the lowest infusion rate to the highest) 15.3 ± 1.3, 24.0 ± 2.8, 39.7 ± 2.8, and 55.3 ± 5.6%. The plasma epinephrine-ALC dose-response relationship is shown in Fig. 7. There was no difference in ALC in the group in which epinephrine was infused at a rate of 9.3 ng·kg⁻¹·min⁻¹ compared with that observed in the group of animals from a previous study (18) in which ALC was determined under baseline conditions. At the higher infusion rates, ALC increased in a dose-related fashion, with the ALC observed at each higher infusion rate being significantly (P < 0.05) greater than that produced by the preceding smaller dose. Figure 7 also shows the relationship between plasma epinephrine concentration and ALC observed in a previous study (18), in which veratrine was administered to massively activate the sympathetic nervous system, and indicates that the slope of the relationship was similar regardless of whether epinephrine was secreted by the animal or was infused.

**DISCUSSION**

In this study the infusion of graded amounts of epinephrine increased ALC in a dose-dependent manner. These results are consistent with previous studies that have reported a dose-response relationship between plasma epinephrine concentration and alveolar transepithelial fluid flux in near-term fetal sheep (5), terbutaline concentration and dome formation (a consequence of active sodium transport) in isolated rat alveolar epithelial type II cells (13), and salmeterol concentration and ALC in isolated nonperfused rat lungs (35). These observations thus indicate that the intact adult canine alveolar epithelium also has the ability to modulate its rate of vectorial sodium and water transport in response to increasing degrees of β₂-adrenoceptor stimulation.

Previously it was concluded that epinephrine released from the adrenal glands was responsible for increasing ALC in a canine model of NPE in which edema was produced by the intracisternal administration of veratrine (18). This conclusion was based on the ability of β₂-adrenoceptor blockade and adrenalectomy to prevent ALC from increasing after veratrine administration and that of epinephrine infused at rates that reproduced the plasma concentrations observed in NPE to produce a similar increment in ALC. Comparison of the slope of the plasma epinephrine concentration-ALC relationship observed after veratrine administration with that produced by epinephrine infusion (Fig. 7) provided further evidence that epinephrine was the adrenal mediator. In this regard, the slopes of the two relationships were identical, indicating that the in-

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**Fig. 5.** Relationship between cardiac output and plasma epinephrine concentration. Data point for lowest plasma epinephrine concentration represents baseline conditions from Ref. 18.

**Fig. 6.** Effect of epinephrine infusions on hematocrit. Number to right of each plot is epinephrine infusion rate (in ng·kg⁻¹·min⁻¹) for that group of experiments. Control (baseline) data are from Ref. 18.

**Fig. 7.** Relationship between alveolar liquid clearance (ALC) and plasma epinephrine concentration for dose-response experiments (this study) and response to massive sympathetic nervous system (SNS) activation (18).
creased plasma epinephrine concentrations that developed after SNS activation could account for the increases in ALC previously observed in the NPE model.

The major objective of this study was to determine whether the increase in ALC observed in the veratrine NPE model represented the maximum response that could be elicited by β₂-adrenergic stimulation. In a previous study, veratrine administration increased plasma epinephrine concentration to an average 7,683 ± 687 pg/ml and ALC to 30.4 ± 1.6%. In this study, it was possible to increase ALC to a greater degree by further increasing plasma epinephrine concentration (Fig. 7). This observation indicates that although there was substantial β₂-adrenergic stimulation during the NPE recovery phase in the previous study, alveolar epithelial sodium and water transport were not maximally stimulated. This observation may have possible clinical relevance in view of recent speculation that β₂-adrenergic agonist therapy might help promote recovery in patients with alveolar edema (1). The efficacy of such therapy would be dependent on the degree to which alveolar epithelial sodium and water transport were upregulated by endogenous mechanisms. The data thus suggest that it may be possible to produce additional increments in ALC during recovery from NPE, with the administration of exogenous β₂-adrenergic agonists having the benefits (35, 42) but not the potential adverse cardiovascular effects of epinephrine (11, 12, 34). In this regard, although the two highest doses of epinephrine produced further increments in ALC, they also resulted in a pattern of progressive circulatory failure (see below).

Although not the intention of this study, the present data do not allow the upper end of the dose-response relationship to be defined. It is thus not clear whether the highest observed rates of ALC were the maximal possible or whether higher rates could have been produced by still higher increments in plasma epinephrine concentration. The decrease in the slope of the plasma epinephrine concentration-ALC relationship observed between the two highest infusion rates suggests, however, that the maximum rate of liquid clearance was being approached. Given the extreme increases in plasma epinephrine concentration that occurred during the highest infusion rate, it does appear safe to conclude, however, that it is unlikely that ALC would reach higher values via stimulation by adrenal epinephrine.

No increase in ALC was observed at the lowest epinephrine infusion rate (9.3 ng·kg⁻¹·min⁻¹), yet cardiac output was increased by ~30%. This comparison suggests that the cardiovascular system is more sensitive to epinephrine than the alveolar epithelial sodium transport system. A significant increase in ALC was produced by the next highest epinephrine dose (88.2 ng·kg⁻¹·min⁻¹), which resulted in an average plasma epinephrine concentration of 1,387 ± 51 pg/ml (Fig. 7). Plasma concentrations of this magnitude or greater have been commonly observed in humans (4, 20, 24, 26–28, 40, 43) and dogs (17, 29) during heavy exercise, suggesting that, under these conditions, circulating epinephrine might play a role in preventing the accumulation of excess liquid in the air spaces. Importantly, the observation that plasma epinephrine concentrations of this magnitude significantly increased ALC indicates that a β₂-adrenergic stimulation of alveolar epithelial sodium and water transport is not solely a manifestation of a severe degree of SNS activation.

The hemodynamic changes observed in this study were typical of those produced by epinephrine administration. At the three lower infusion rates, epinephrine increased cardiac output (Figs. 4 and 5), but Pa remained relatively constant (Fig. 3). Thus systemic vascular resistance must have fallen. This pattern is consistent with epinephrine's action as a systemic vasodilator (15). At higher concentrations, however, epinephrine produces vasoconstriction. Thus, at the highest infusion rate, Pa initially increased (Fig. 3), despite a reduction in cardiac output (Figs. 4 and 5). During the course of the observational period, however, Pa progressively decreased to values less than baseline, as has previously been observed during epinephrine infusions of this magnitude (12, 34). The reduction in Pa may have resulted from the observed progressive fall in cardiac output (Fig. 4). The latter may reflect the toxic effects of high concentrations of catecholamines on the myocardium (30, 31) and possibly a decrease in circulating blood volume (12). Epinephrine also increased hematocrit in a dose-dependent manner. Similar increases in hematocrit are observed after massive SNS activation in the dog and have been found to result from an adrenergic-mediated release of erythrocytes from the spleen (21).

In summary, the results of this study indicate that epinephrine increases ALC in a dose-dependent manner. Analysis of this relationship provided additional evidence that epinephrine mediated the increased ALC observed during recovery from NPE and that ALC was not maximally stimulated under these conditions. The latter observation suggests that further gains in the rate of resolution of this form of edema might be achieved using β₂-adrenergic agonists having the benefits, but not the adverse effects, of epinephrine. Finally, these results also indicate that ALC can be increased under conditions of less intensive SNS activation and suggest that alveolar epithelial sodium transport might be stimulated under such conditions as heavy exercise.

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