Effect of epinephrine on alveolar liquid clearance in the rat

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Charron, Paul D., J. Phillip Fawley, and Michael B. Maron. Effect of epinephrine on alveolar liquid clearance in the rat. J. Appl. Physiol. 87(2): 611–618, 1999.—Endogenous epinephrine has been found to increase alveolar liquid clearance (ALC) in several pulmonary edema models. In this study, we infused epinephrine intravenously for 1 h in anesthetized rats to produce plasma epinephrine concentrations commonly observed in this species under stress. ALC by mass balance. Epinephrine increased ALC from 31.5 ± 3.2 to 48.9 ± 1.1 (SE)% of the instilled volume (P < 0.05). The increased ALC was prevented by either propranolol or amiloride. To determine whether ALC returns to normal after plasma epinephrine concentration normalizes, we measured ALC 2 h after stopping an initial 1-h epinephrine infusion and found ALC to be at baseline values. Finally, to determine whether desensitization of the liquid clearance response occurs, we evaluated the effects of both repeated 1-h infusions and a continuous 4-h infusion of epinephrine on ALC and found no reduction in ALC under either condition. We conclude that epinephrine increases ALC by stimulating β2-adrenergic receptors and sodium transport, that the increase is reversible once plasma epinephrine concentration normalizes, and that desensitization of the ALC response does not appear to occur after 4 h of continuous epinephrine exposure.

IT IS NOW generally accepted that the removal of excess water from the alveolar air spaces involves the active transport of sodium followed by the passive movement of water across the alveolar epithelium (36). Specifically, sodium is considered to enter the alveolar epithelial type II cell through multiple specialized apical pathways and then be pumped out of the basolateral side by the enzyme Na1-K1-ATPase. Recent work has suggested that the alveolar epithelial type I cell may provide an important pathway for the resultant osmotically driven water flux (8). Although the mechanisms by which this process is regulated are not completely understood, β2-adrenergic agonists (e.g., terbutaline, salmeterol, epinephrine) have been found to increase the rate of alveolar epithelial sodium and water transport in most species, including humans (4–7, 9, 12–14, 32, 33, 35, 37, 38). These observations have led to the proposal that the administration of β2-adrenergic agonists might be used clinically to accelerate the rate of edema resolution in patients with pulmonary edema (2). More recently, the rate of fluid reabsorption from the air spaces [alveolar liquid clearance (ALC)] has been found to be increased in animal models of pulmonary edema produced by neurological insults (18) and sepsis (29) and under conditions of hemorrhagic shock (23, 28). These observations suggest that epinephrine released from the adrenal glands might play a role in enhancing edema resolution in patients with edema resulting from these stimuli.

The alveolar epithelial fluid reabsorptive response to epinephrine, however, is incompletely understood. For example, it is not known how quickly ALC returns to normal once plasma epinephrine concentration is normalized. This is an important question, because of the possibility that any benefit derived from endogenous epinephrine might be closely linked to the maintenance of elevated plasma epinephrine concentrations. Second, inasmuch as desensitization is a commonly observed feature of β2-adrenergic receptors (3, 11, 24), the long-term effectiveness of epinephrine (or other β2-agonists for that matter) in clearing fluid from the air spaces is unknown. Accordingly, the major objectives of this study were to determine whether epinephrine-stimulated rates of ALC return to baseline levels after plasma epinephrine concentration is normalized, whether initial exposure to an elevated plasma epinephrine concentration depresses the ability of a subsequent epinephrine exposure to increase ALC, and whether ALC remains increased after prolonged exposure to epinephrine.

METHODS

Animal preparation. Seventy-five male Sprague-Dawley rats (mean weight 376 g; Zivic-Miller) were anesthetized with pentobarbital sodium (80 mg/kg ip), with the anesthetic being supplemented as needed. Body temperature was monitored by using a rectal thermistor and maintained by using a water-perfused heating pad. A tracheal cannula was placed in the rat’s airway via a tracheotomy and connected to a mechanical ventilator. The lungs were ventilated (tidal volume 3.5 ml, respiratory rate 54 breaths/min) by using 100% O2. Peak inspiratory pressure was 8.7 ± 0.8 (SD) Torr, and expiratory pressure was set at 2.0 Torr by using a water-overflow system. Both femoral veins and the right femoral artery were cannulated with catheters made of polyethylene tubing (PE-50). The venous catheters were used for drug administration, and the arterial catheter was used to monitor arterial pressure and heartrate and for blood sampling. Blood samples (0.3 ml) were drawn at 0.5-h intervals for blood-gas analysis by using a Radiometer system. Blood gases were adjusted by altering the ventilatory parameters and infusing sodium bicarbonate as necessary and were under baseline conditions: PO2 485 ± 62 (SD) Torr, PCO2 36.8 ± 3.9 Torr, pH 7.41 (range 7.31–7.47). The animals were allowed to
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Determination of ALC. ALC was determined by using the principle of mass balance (14). Briefly, 3 ml/kg of a Ringer lactate solution (Baxter Healthcare, Deerfield, IL) containing 5% BSA were instilled into the lungs. The solution was adjusted with NaCl to be isotonic with rat plasma (290–300 mosmol/kg). On the morning of the experiment, 0.25 g BSA (Sigma Chemical, St. Louis, MO) was dissolved in 5 ml of the Ringer solution to produce the instillate. The rat was placed at a 45° angle (head elevated), and a polyethylene catheter (PE-10) was inserted through a port in the tracheal cannula into the lungs. The 5% BSA solution was instilled into the lungs at a rate of 0.10 ml/1.5 min. The alveolar instillate was left in the lungs for 1 h beginning at the completion of the instillation. At this time, the rat was euthanized with an overdose of pentobarbital sodium, the lungs were removed, and the remaining fluid was aspirated for the analysis of albumin concentration by refractometry (American Optical, Buffalo, NY). The refractometer was calibrated by using a series of albumin standards (Sigma Chemical). Because the initial volume of the instilled solution and the initial and final albumin concentrations were known, ALC could be determined by by using the following mass-balance equation

\[ \text{ALC} = \left(1 - \frac{A_{\text{final}}}{A_{\text{initial}}}\right) \times 100 \]

where ALC is expressed as the percentage of the instilled fluid that left the air spaces during the 1-h observation period and Alb and Alb are, respectively, the initial and final albumin concentrations of the instillate.

Epinephrine infusion and analysis. (--) Epinephrine (Sigma Chemical) was dissolved in saline acidified with 0.001 N HCl and was infused intravenously at a rate of 181 ng·kg\(^{-1}\)·min\(^{-1}\) (0.013 ml/min). This dose was selected to produce plasma epinephrine concentrations within the range observed in the rat after such stressors as immobilization, tail or foot shock, and cold and heat exposure (10, 15–17, 39). For control experiments, the acidified saline vehicle was administered at the same rate as that used in the rats receiving epinephrine. Plasma catecholamine concentrations were determined by HPLC as previously described (19).

Experimental studies. Three studies were conducted by using this preparation. The purpose of the first study was to determine whether the infused dose of epinephrine increased ALC and, if so, to confirm that the increase was mediated by β-adrenergic receptors and amiloride-sensitive sodium channels. This study was also designed to provide measurements of ALC at 1 h that would serve as a basis of comparison for similar determinations made at later times in study 2. In these experiments, either epinephrine or acidified saline was infused for 1 h immediately after airway fluid instillation. At the end of the hour, the rats were euthanized, and ALC was determined. The following groups of rats were studied. In six animals each, either saline or epinephrine was infused. These experiments were repeated (5 animals each) in the presence of the sodium-channel blocker, amiloride (10\(^{-3}\) M dissolved in the instillate; Sigma Chemical). In five animals, propranolol (0.4 mg iv; Sigma Chemical), a β-adrenergic receptor antagonist, was administered before the epinephrine infusion was started. Efficacy of the receptor blockade was determined in each experiment by administration of the β-adrenergic agonist, isoproterenol (0.08 µg iv, Sigma Chemical). The absence of tachycardia after isoproterenol injection was interpreted as indicating lack of β-adrenergic receptor blockade. To verify that propranolol had no effect on baseline ALC, an additional experiment was done in which we administered propranolol to a rat that received a saline, rather than an epinephrine, infusion. In two rats, ouabain (5 × 10\(^{-4}\) M dissolved in the instillate and 4 µg iv; Sigma Chemical) was administered before saline infusion to evaluate the effect of Na\(^{+}\)-K\(^{-}\)-ATPase inhibition on ALC under baseline conditions.

RESULTS

Study 1. Plasma epinephrine concentration was significantly elevated in animals in which epinephrine
was infused but not in those that received the saline vehicle (Table 1). In control rats, alveolar fluid albumin concentration increased from 4.91 ± 0.07 (SE) to 7.24 ± 0.30 g/dl during the 1-h observation period. In contrast, albumin concentration increased to a greater degree (4.99 ± 0.03 to 9.78 ± 0.23 g/dl) in rats in which epinephrine was infused during this period. Under baseline conditions, ALC was 31.5 ± 3.2% of the instilled volume, a value similar to that reported previously for this species (14). Infusion of epinephrine increased ALC by ~55% to 48.9 ± 1.1% of the instilled volume (P < 0.05; Fig. 2).

The increase in ALC was prevented by the administration of either amiloride or propranolol (P < 0.05; Fig. 2). In control animals, amiloride administration decreased ALC by ~28% (P < 0.05; Fig. 2). ALC after epinephrine infusion was reduced ~50% when the measurements were repeated in the presence of amiloride. The amiloride-sensitive fractions of the baseline and epinephrine-stimulated rates of ALC were, respectively, 28 and 88%. Ouabain decreased baseline ALC by 56% (ALC = 14.0 ± 7.4%; P < 0.05 compared with baseline values). In animals infused with epinephrine in the presence of propranolol, the average baseline plasma epinephrine concentration was elevated and in the range observed after epinephrine infusion, with a further increase being observed after epinephrine administration. The reason for the elevated baseline concentration is not clear but could be related to either the administration of propranolol or the use of isoproterenol to test the effectiveness of the β-adrenergic-receptor blockade. In any event, no increase in ALC was observed in the propranolol group even though the average plasma epinephrine concentration was elevated ~1,000 pg/ml over that observed when epinephrine was administered alone. Propranolol did not alter baseline ALC (26.2%), as has been previously observed by Jayr et al. (14). Epinephrine infusion did not alter arterial pressure or heart rate, and no increase in pulmonary arterial pressure was observed in the two animals in which this pressure was monitored during the experiment. Epinephrine infusion did not produce major changes in plasma norepinephrine concentration in any of the groups (Table 1).

### Table 1. Plasma catecholamine concentrations in study 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Epinephrine, pg/ml</th>
<th>Norepinephrine, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 1 h</td>
<td>Baseline 1 h</td>
</tr>
<tr>
<td>Control</td>
<td>0 ± 0</td>
<td>222 ± 67 245 ± 41</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>0 ± 0</td>
<td>1,888 ± 286</td>
</tr>
<tr>
<td>Epinephrine + propranolol</td>
<td>1,710 ± 962 2,968 ± 662</td>
<td>238 ± 72 220 ± 56</td>
</tr>
<tr>
<td>Epinephrine + amiloride</td>
<td>0 ± 0</td>
<td>1,098 ± 288</td>
</tr>
<tr>
<td>Control + amiloride</td>
<td>85 ± 85</td>
<td>275 ± 36 213 ± 28*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 compared with baseline value.
saline was administered during both infusion periods (saline/saline group). ALC (18.3 ± 0.8%) was not increased in rats in which ALC was measured between the second and third hours after the epinephrine infusion was stopped (epinephrine/saline group). In these animals, plasma epinephrine concentration was elevated during the epinephrine infusion but had returned to baseline values by the time ALC was measured (Table 2). In contrast, ALC was significantly turned to baseline values by the time ALC was measured in plasma epinephrine concentration was elevated in the epinephrine/saline group. A similar increase (P < 0.05) in ALC (37.3 ± 3.6%) was observed in the group that received epinephrine during both infusion periods (epinephrine/epinephrine group).

Study 3. Plasma catecholamine concentrations for the 4-h infusion studies are shown in Table 4. Plasma epinephrine concentrations were elevated in the epinephrine- but not the saline-infusion groups. No changes in plasma norepinephrine concentration occurred. The infusion of epinephrine for 4 h increased ALC to a similar degree to that observed in the saline/epinephrine group (study 2) in which epinephrine was infused during only the last hour of the 4-h study period (Fig. 4).

Although within a given study epinephrine increased ALC to a similar degree with respect to the baseline rate of clearance, both baseline and epinephrine-stimulated ALCs were reduced essentially the same absolute amount when measured between hours 3 and 4 compared with similar measurements made during the first hour (Fig. 5).

**DISCUSSION**

The infusion of epinephrine produced significant increases in ALC (Fig. 2) that were mediated by β-adrenoceptors and an increase in alveolar epithelial sodium transport, because the increased ALC could be blocked by either the administration of propranolol (a nonspecific β-adrenoceptor antagonist) or amiloride (a sodium-channel blocker). These results were consistent with previous observations showing that the increase in ALC produced by epinephrine in the dog could be attenuated by the specific β2-adrenoceptor antagonist ICI-118551 (18) and those indicating that the increased clearance produced by β2-adrenergists in general can be reduced by inhibitors of sodium transport (13, 14, 33, 35, 37). The plasma epinephrine concentrations (~600–2,000 pg/ml) eliciting this response were well within the range commonly observed in rats subjected to a variety of stressors, including immobilization, tail or foot shock, hypothermia, and hyperthermia (10, 15–17, 39), and were not of a sufficient magnitude to produce changes in arterial or pulmonary arterial blood pressure or heart rate. This analysis suggests that an increase in alveolar epithelial sodium transport may be a commonly occurring consequence of sympathetic activation and is consistent with our previous observations that plasma epinephrine concentrations of a magnitude (1,387 pg/ml) occurring during heavy exercise increased ALC in dogs (21).

A major objective of this study was to determine whether the maintenance of a stimulated ALC by epinephrine requires the continued presence of an elevated plasma epinephrine concentration. To answer this question, we compared ALC estimates determined between the second and third hour after stopping an initial 1-h infusion of epinephrine (epinephrine/saline group in study 2) with those obtained in rats in which only saline had been infused during the initial 1-h period (saline/saline group) and found ALC to be at baseline values in both groups (Fig. 3). This observation was not the result of an inability of the rat to respond to epinephrine during the period in which ALC was measured, because epinephrine increased ALC in both the saline/epinephrine and epinephrine/epinephrine groups at this time (Fig. 3). Although we did not measure ALC during the first hour in these studies, the observation that ALC was increased when epinephrine was infused during this period in the epinephrine infusion group in study 1 indicated that ALC was most likely elevated in the epinephrine/saline group at this
time. These data thus indicate that the elevated ALC returned to baseline values after plasma epinephrine concentration became normalized. This observation may have clinical significance. In this regard, endogenous epinephrine has been shown to be responsible for increasing ALC in several animal models of pulmonary edema (18, 23, 29). Our data thus suggest that patients with pulmonary edema associated with elevated plasma epinephrine concentrations might exhibit an increased ALC for only as long as plasma epinephrine concentration remains elevated.

Inasmuch as agonist-promoted desensitization is a commonly observed characteristic of the β₂-adrenergic receptor signal-transduction system (3, 11, 24), a second major objective was to determine whether epinephrine reexposure resulted in a smaller increase in ALC compared with that observed during the initial infusion. The observation that ALC in the epinephrine/epinephrine group of study 2 was no different from that observed in the saline/epinephrine group (Fig. 3) indicated that the repeated administration of epinephrine did not diminish the response. Because there was a 2-h period between the two epinephrine infusions, it is conceivable, however, that any desensitization that might have occurred may have reversed by the time of the second infusion. Accordingly, study 3 was designed to evaluate the effects of a 4-h continuous epinephrine infusion. In these experiments, the increase in ALC measured during the last hour of epinephrine infusion was no different from that observed in the saline/epinephrine group (study 2), in which epinephrine was infused during only the last hour of the 4-h study period (Fig. 4).

The observation that ALC was not reduced after repeated or continuous exposure to epinephrine may at first seem surprising, because desensitization is a well-described regulatory mechanism of β₂-adrenoceptors (3, 11, 24). There are a number of possible explanations, however, for our observations. First, it is possible that alveolar epithelial type II β₂-adrenoceptors might not undergo significant desensitization. In this regard, the degree of β₂-adrenoceptor desensitization that may develop within different cell types within the lung appears to be cell specific (22, 25, 26). For example, McGraw and Liggett (22) observed that human airway smooth muscle cells exhibited very little desensitization in response to β₂-adrenoceptor stimulation compared with that observed in mast cells and that this difference was related to heterogeneity in the expression of β-adrenergic-receptor kinase, an enzyme responsible for phosphorylating the receptor and producing desensitization. Alternatively, it is possible that some degree of desensitization might have occurred but may not have been manifested as a reduction in ALC, because there may not be a direct correlation between receptor activation and the consequent physiological response (increase in ALC) (25). Finally, it is also conceivable that our experimental design may not have been optimized to observe reductions in ALC that could have been produced by all of the diverse regulatory mechanisms that produce receptor desensitization. In this regard, desensitization is a complex phenomenon involving a number of regulatory processes occurring over varying time frames (3). The most rapid event (within seconds to minutes) is phosphorylation of the receptor, which results in an uncoupling of the receptor from the stimulatory guanine nucleotide-binding pro-

| Table 4. Plasma catecholamine concentrations in study 3 |
|-------------------|-------------------|-------------------|
|                   | Epinephrine, pg/ml | Norepinephrine, pg/ml |
|                   | Baseline | 2 h | 4 h | Baseline | 2 h | 4 h |
| Control           | 45 ± 45 | 184 ± 95 | 128 ± 40 | 144 ± 70 | 115 ± 86 | 238 ± 83 |
| Epinephrine       | 80 ± 29 | 1249 ± 208* | 1629 ± 423* | 172 ± 43 | 207 ± 41 | 217 ± 27 |

Values are means ± SE. *P < 0.05 compared with baseline value.

Fig. 4. Effects of 4-h epinephrine infusion on ALC. Saline (4 hr), 4-h saline infusion; Epi (4 hr), 4-h epinephrine infusion; Epi (last hr), epinephrine infused only during last hour of 4-h experiment (data from saline/Epi group, Fig. 3). *P < 0.05 compared with saline (4 hr) group.

Fig. 5. Effect of time on determination of ALC under baseline and epinephrine stimulated conditions. *P < 0.05 compared with respective determination made during 1st hour.
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Protein Gs (3, 11, 24). Because epinephrine was infused in all experiments for at least 1 h, it is conceivable that some degree of receptor phosphorylation and desensitization might have occurred soon after the infusion was begun but was not observed, because the ALC measurement reflects the sum of all of the liquid clearance that occurred during the entire 1-h infusion period. Over a longer period (h), a net loss of cellular receptor binding sites (downregulation) can occur (3, 11, 24). If this process requires more than 4 h in the alveolar epithelial type II cell, our results would not have reflected this event. In any event, the maintained ability of epinephrine to stimulate the clearance of excess fluid from the air spaces for as long as 4 h suggests that either the endogenous release of epinephrine or the administration of exogenous β2-adrenergic agonists may be of therapeutic benefit in patients recovering from pulmonary edema.

An unanticipated observation was that the ALC estimate was smaller when measured between the third and fourth hours compared with the value obtained during the first hour. Because the absolute reduction was similar under both baseline conditions and after stimulation with epinephrine (Fig. 5), the reductions appear to be related to factors other than the ability of the alveolar epithelium to respond to β-adrenergic stimulation. Although the reason for the diminished responsiveness is not clear, the reduced ALCs could have mechanistically resulted from either one factor or a combination of reduced sodium inflow into the type II cell through apical sodium channels, a reduction in Na+-K+-ATPase activity, or a reduced transepithelial water flux. Because ALC has been shown to remain constant in isolated perfused rat lungs for up to 5 h (34), it is likely that the time-related decreases in ALC observed in the intact animal relate to some change in the animal’s physiological status that occurred over time. For example, because ALC in the rat is depressed by some anesthetics (30), it is possible that the decrement might have reflected a progressively developing anesthetic effect. Alternatively, because atrial natriuretic factor has been shown to decrease alveolar transepithelial sodium transport (27), it is conceivable that ALC could have been reduced if plasma atrial natriuretic factor concentration (or that of other hormones that might affect sodium transport) had increased later in the experiment. Other possible explanations include hyperoxia and hypoxia. Because the rats were ventilated with 100% O2, it is conceivable that the reduced ALCs might have resulted from an increase in alveolar epithelial protein permeability. The latter could have reduced the measured increase in instillate protein concentration, causing ALC to be underestimated. This possibility seems to be unlikely; however, in view of observations by Royston et al. (31) indicating that, in the rat, alveolar epithelial permeability to even relatively small solutes ([99mTc-labeled diethylene triamine pentaacetic acid) is not increased after 24 h of ventilation with 100% O2. Finally, because hypoxia has been shown to decrease sodium transport in isolated rat alveolar type II cells (20), we considered the possibility that the reduced ALCs resulted from a derangement in blood gases. An analysis of blood-gas data in the 1- and 4-h experiments, however, revealed no differences in PO2, PCO2, or pH in the animals infused with either saline or epinephrine (data not shown). Regardless of the cause, however, these data indicate the importance in experimental studies of comparing stimulated rates of ALC with baseline measurements made at similar time points.

Although epinephrine was infused at the same weight-adjusted rate in each experiment, the measured plasma epinephrine concentration varied by as much as threefold in some groups (Tables 1, 2, and 4). A similar degree of variability was also sometimes observed within a given group and in individual rats in which multiple infusions of epinephrine were made (epinephrine/epinephrine group of study 2). The reason for this variation is not clear and is surprising in view of the consistency in plasma epinephrine concentrations that we previously observed during epinephrine infusion in dogs (21). Measurements of the infusate epinephrine concentration in some of the experiments in which such variation was observed indicated that the variability could not be explained by differences in the amount of epinephrine infused or our ability to accurately measure epinephrine concentration. Nor do the variable plasma epinephrine concentrations appear to be the result of inadvertent dilution of the sample, because the plasma norepinephrine concentrations did not exhibit a similar pattern. The variability thus appears to relate to inherent individual differences in plasma epinephrine clearance and/or compartmental distribution. With respect to the latter, at plasma concentrations similar to those we produced, approximately one-half of the epinephrine carried in the blood of the rat has been found to be localized within erythrocytes (1) and would thus not be measured in a plasma assay. It is thus conceivable that differences in the erythrocyte-plasma epinephrine distribution, if they were to occur, could result in variable plasma epinephrine concentrations. In any event, the observed degree of variation did not appear to be sufficiently large as to produce differences in ALC. Supportive of the latter conclusion are our previous observations indicating that average plasma epinephrine concentration of either 7,683 or 15,737 pg/ml increased ALC to the same degree in dogs (21).

In conclusion, we found that 1) elevated plasma epinephrine concentrations within the range observed in rats subjected to a variety of stressors increased ALC via a β-adrenoceptor-mediated increase in alveolar epithelial sodium transport, 2) the maintenance of the increased ALC requires the presence of an elevated plasma epinephrine concentration, and 3) the rate of liquid clearance observed after 4 h of epinephrine infusion was no different from that observed after a 1-h infusion. These results may also be pertinent to the potential clinical use of exogenous β2-agonists as therapy to promote resolution of pulmonary edema (2, 32).
Finally, although these data suggest that patients recovering from forms of pulmonary edema that are accompanied by increased plasma epinephrine concentrations might exhibit an increased ALC for as long as epinephrine concentrations are elevated, additional studies examining a longer time course of epinephrine exposure will be required to more fully address this issue.

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