Lidocaine Induces a Reversible Decrease in Alveolar Epithelial Fluid Clearance in Rats

Marc Laffon, M.D.,* Christian Jayr, M.D.,† Pascal Barbry, Ph.D.,‡ Ybing Wang, M.D.,§ Hans G. Folkesson, Ph.D.,‖ Jean-Francois Pittet, M.D.,# Christine Clerici M.D.,** Michael A. Matthay, M.D.††

Background: **Lidocaine is widely used in patients with acute cardiac disorders and has also been recently implicated as a possible cause of pulmonary edema after liposuction. The objective of this study was to assess the effect of lidocaine on alveolar fluid clearance, the primary mechanism responsible for the resolution of alveolar edema.**

Methods: **Alveolar fluid clearance was measured in 29 ventilated rats using our well-validated method over 1 h using a 5% albumin solution instilled into the distal air spaces of the lung. Lidocaine was added to the instilled albumin solution (10^{-5} M) or administered intravenously at a dose estimated to achieve a clinically relevant plasma concentration of 10^{-5} M. Standard agonists and antagonists were used to determine the effect of lidocaine on alveolar fluid clearance. To determine whether lidocaine acted predominantly on the apical or basal surface, we also used QX314, lidocaine *n*-ethyl bromide quaternary salt, an analog of lidocaine, which is unable to cross the alveolar epithelium. The effect of lidocaine on the apical epithelial sodium channel transfected in *Xenopus* oocytes was also studied.**

Results: **Alveolar or intravenous lidocaine decreased alveolar fluid clearance by 50%, an effect that was reversible with the β_2 agonist, terbutaline. Lidocaine acted predominantly on the basal surface of the epithelium because *n*-ethyl bromide quaternary salt decreased alveolar fluid clearance only when it was given intravenously and because lidocaine did not inhibit the apical epithelial sodium channel when expressed in oocytes.**

Conclusions: **Lidocaine decreased alveolar fluid clearance by 50%, an effect that may have major clinical implications in the care of patients with cardiac disease or during the perioperative period in some patients. Importantly, the effect of lidocaine was completely reversible with β_2-adrenergic therapy.**

SEVERAL studies have demonstrated that active ion transport is the primary mechanism for the clearance of pulmonary edema fluid from the distal air spaces of the lung. During pathologic conditions, alveolar fluid clearance can be up-regulated by catecholamine-dependent and -independent pathways.[1–5] Sodium is transported by alveolar epithelial cells by two transport mechanisms. The initial step is the uptake of sodium by the apical amiloride-sensitive epithelial sodium channel (ENaC). The second step is active extrusion of sodium from epithelial cells through the basolateral membrane by sodium-potassium ATPase (NaKATPase). Chloride uptake then follows along an electrochemical gradient across the epithelium, probably by a transcellular route, and water is absorbed osmotically because of the mini-osmolar salt gradient, probably through transcellular water channels called aquaporins.[6] β-adrenergic agonists can increase net salt and fluid absorption by cyclic adenosine monophosphate–dependent mechanisms that involve both membrane and transcriptional effects.[7] Recently, Rezaiguia-Delclaux *et al.*[8] reported that the inhalation anesthetics, such as halothane and isoflurane, induce a reversible decrease in alveolar epithelial fluid clearance in rats. In addition, halothane decreased the activity of amiloride-sensitive sodium channels and NaKATPase activity in alveolar type II cells.[9] However, there are currently no data on the effect of local anesthetics on alveolar epithelial fluid transport.

Lidocaine is used clinically as an antiarrhythmic agent in patients with ischemic heart disease, as a local anesthetic during bronchoscopy, and locally during a variety of different surgical procedures. In humans, Knowles *et al.*[10,11] reported that lidocaine decreased nasal and tracheal transepithelial ion transport, an effect that was explained by blockade of sodium or potassium ion transport. Lidocaine induces a blockade of voltage-gated sodium channels in neural nerves[12,13] and blocks basolateral potassium channels in frog alveolar epithelium.[14] As discussed in detail by Dawson *et al.*,[15] basolateral potassium channels play a role in the regulation of vectorial sodium and chloride transport. Interestingly, several recent studies have shown that lidocaine may be a possible cause of pulmonary edema in patients undergoing liposuction.[16–18] These data suggested that lidocaine could inhibit alveolar epithelial ion and fluid transport mechanisms and lower the threshold for the development of pulmonary edema.

The first objective of the current *in vivo* rat study was to assess the effect of clinically relevant concentrations of lidocaine on alveolar epithelial fluid transport. Because lidocaine decreased alveolar fluid clearance, the second objective was to determine the mechanism responsible for the lidocaine-induced decrease in alveolar fluid transport as well as to determine if the inhibitory effect of lidocaine could be overcome with β-adrenergic therapy. Moreover, to determine whether the effect of lidocaine was predominantly on apical or basal alveolar fluid transport, we used *n*-ethyl bromide quaternary salt

392

(QX-314), an analog of lidocaine that cannot easily cross the cell membrane, and the effect of lidocaine was also tested on the function of the primary ENaC expressed in oocytes.

Materials and Methods

Male Sprague-Dawley rats weighing 300–350 g were studied. The rats were housed in air-filtered, temperature-controlled rooms. The University of California–San Francisco Animal Research Committee approved the protocol for these studies.

Surgical Preparation and Ventilation

The rats were anesthetized with pentobarbital (60 mg/kg administered intraperitoneally), and anesthesia was maintained with 30 mg/kg body weight of pentobarbital sodium every 1 h. An endotracheal tube (PE-220; Clay Adams, A Becton-Dickinson Company, Parsippany, NJ) was inserted through a tracheotomy. Pancuronium bromide (0.3 mg · kg body weight^{-1} · h^{-1}; Pavulon®; Organon Inc., West Orange, NJ) was given for neuromuscular blockade. A catheter (PE-50; Clay Adams) was inserted into the carotid artery to monitor systemic arterial pressure and to obtain blood samples. A second catheter was inserted into the jugular vein for intravenous drug administration. The rats were maintained in the right lateral decubitus position during the experiments to facilitate fluid deposition into the right lung. They were ventilated with a constant-volume pump (Harvard Apparatus, Dover, MA) with an inspired oxygen fraction of 1.0 and peak airway pressures of 8–12 cm H_2O, supplemented with positive end-expiratory pressure of 3 cm H_2O. The respiratory rate was adjusted to maintain the arterial carbon dioxide tension between 35 and 40 mmHg. Body temperature was kept constant at 38°C with a thermostatically controlled pad.

Preparation of the Instillate

A 5% albumin (bovine serum albumin; Sigma Biochemical Co., St. Louis, MO) solution was prepared using Ringer lactate and adjusted with NaCl to be isosmolar with the rat's circulating plasma, as in our previous studies.[2,8] Anhydrous Evan blue dye (0.5 mg; Aldrich Chemical Company Inc., Milwaukee, WI) was added to the albumin solution to confirm the location of the instillate at the end of the study, and 1 μCi of ^{125}I-labeled human serum albumin (Frosst Laboratories, Montreal, Quebec, Canada) was added to the albumin solution to measure the protein permeability of the alveolar epithelium (see below). A sample of the instilled solution was saved for total protein measurement to be used to calculate alveolar fluid clearance (see Measurements), radioactivity counts, and the water-to-dry weight ratio measurements so that the dry weight of the protein

solution could be subtracted from the final lung water calculation.

General Protocol

In all experiments, after the surgery, heart rate and systemic blood pressure were allowed to stabilize for 1 h. Then a vascular tracer, 1 μCi of ^{131}I-labeled human albumin (Frosst Laboratories), was injected intravenously to calculate the flux of the plasma protein into the lung interstitium. Thirty minutes later, the 5% albumin instillate solution with 1 μCi of ^{125}I-labeled albumin (alveolar tracer) was instilled into the right lower lobe to calculate the flux of protein from the air spaces into the circulating plasma. The instillate solution (3 ml/kg) was instilled over 20 min using a 1-ml syringe and a polypropylene tube (0.5 mm ID) advanced into a wedged position in the right lung. Mechanical ventilation was uninterrupted during instillation, and 1 h after instillation, the abdomen was opened and the rats were exsanguinated by transecting the abdominal aorta. Urine was obtained for radioactivity counts by puncturing the bladder. The lungs were removed through a median sternotomy. An alveolar fluid sample from the distal airspaces (0.1–0.2 ml) was obtained by gently passing the sampling catheter (PE-50 catheter, 0.5 mm ID) into a wedged position in the instilled area of the right lower lobe and aspirating. After centrifugation of the samples, total protein concentration and radioactivity of the sampled fluid were measured. The right and left lungs were homogenized separately for the water-to-dry weight ratio measurements and radioactivity counts.

Specific Protocols

All rats were studied for 1 h and then killed and processed as described in the general experimental protocol.

Group 1: Control Experiments. In group 1 (n = 6), after the baseline period, 3 ml/kg body weight of the 5% albumin solution with ^{125}I-labeled human serum albumin were instilled into one lung.

Group 2: Effect of Intraalveolar or Intravenous Lidocaine. To study the effects on intraalveolar lidocaine, in group 2 (n = 11) we added 10^{-5} M lidocaine (Sigma Biochemicals) to the instilled 5% albumin solution (n = 5). In the studies of the effects of intravenous lidocaine (n = 6), the rats received a bolus of 2 mg/kg lidocaine intravenously followed by a continuous intravenous infusion at a rate of 2 mg · kg^{-1} · h^{-1} lidocaine until the end of the experiment. The intravenous lidocaine infusion started 30 min before the instillation of 5% albumin solution. The intravenous infusion at this dose results in a lidocaine concentration of 10^{-5} M in the plasma, which is a clinically relevant concentration when lidocaine is used intravenously.[19] After the baseline period, 3 ml/kg body weight of the 5% albumin solution was instilled into one lung.

Group 3: Effect of β-adrenergic Agonist Stimulation on the Lidocaine-induced Decrease in Alveolar Fluid Clearance. To study the effects of β-adrenergic stimulation in the presence of lidocaine, in group 3 (n = 8) we instilled the rats with terbutaline as follows. Terbutaline (10^{-4} M; Sigma) and lidocaine (10^{-5} M) were added to the instilled 5% albumin instillate (n = 4). Terbutaline (10^{-4} M; n = 4) without lidocaine was added to the 5% albumin instillate and instilled in a separate control group.

Group 4: Effect of Instilled Lidocaine on Amiloride Inhibition. Normally, amiloride reduces alveolar fluid clearance in rats by 40–60%.[6] To study the effect of lidocaine on amiloride inhibition, in group 4 (n = 8) we instilled the rats with amiloride as follows. Amiloride (10^{-3} M; ICN Biochemicals, Inc., Costa Mesa, CA) and lidocaine (10^{-5} M) were added to the instilled 5% albumin solution (n = 4). Amiloride (10^{-3} M; n = 4) without lidocaine was added to the 5% albumin instillate and instilled in a separate control group.

Group 5: Effect of Instilled and Intravenous Lidocaine *n*-Ethyl Bromide Quaternary Salt. We hypothesized that lidocaine may decrease sodium transport by inhibition of basolateral potassium channels. To test this hypothesis, in group 5 (n = 6) we used QX-314 (Sigma), a quaternary analog of lidocaine that blocks voltage-gated sodium channels and potassium channels, similar to the effects described for lidocaine.[13,14] Since QX-314 is permanently positively charged, it is not able to cross the cell membranes. If QX-314 is administered intravenously, it can accumulate in the lung interstitium on the basal side of the alveolar epithelial cells; if QX-314 is administered into the alveolar space, it should remain on the apical side of the alveolar epithelium because the alveolar epithelium is very tight,[20] thus making it difficult for QX-314 to cross the tight epithelial junctions. Therefore, theoretically QX-314 could inhibit basolateral potassium channels if given intravenously, but it does not have this effect when administered in the alveolar spaces.

To study the intraalveolar effects of QX-314, we added it (10^{-5} M) to the instilled 5% albumin solution (n = 3). In the studies of the effect of intravenous QX-314 (n = 3), the rats received the same dose previously used with lidocaine, *i.e.*, a bolus 2 mg/kg QX-314 intravenous dose followed by a continuous intravenous infusion at a rate of $2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, until the end of the experiment. The intravenous QX-314 infusion started 30 min before the instillation of 5% albumin solution.

Measurements

Hemodynamics, Pulmonary Gas Exchange, and Protein Concentration. Systemic arterial, central venous, and airway pressures were continuously measured. Arterial blood gases were measured at the beginning and the end of the studies. Samples from the instilled protein solution, from the final distal air space fluid, and from the initial and final blood were collected to measure total protein concentration with an automated analyzer (AA2 Technicon, Tarrytown, NY).

Protein Permeability across Endothelial and Epithelial Barriers. Two different methods were used to measure the bidirectional protein permeability across the lung endothelial and epithelial barriers.[3,8] The first method measures residual ^{125}I-albumin (the air space protein tracer) in the lungs and the accumulation of ^{125}I-albumin in the plasma. The second method measures accumulation of ^{131}I-albumin (the vascular protein tracer) in the air spaces of the lungs.

For measuring the flux of the alveolar tracer protein, ^{125}I-albumin, from the lung, several measurements were necessary. The total radioactivity instilled into the lungs (^{125}I-albumin, counts per minute per gram) was calculated by multiplying the radioactivity in aliquots of the instillate by the volume instilled. The residual quantity of ^{125}I-albumin in the lungs at the end of the experiment was calculated by the average of the duplicate radioactivity counts from the lung homogenates from the instilled lung multiplied by the volume of the lung homogenate. To measure the passage of ^{125}I-albumin from the alveolar spaces into the blood, the radioactivity counts in the plasma samples were multiplied by the plasma volume (milliliter). The plasma volume was calculated by the following relation:

To measure the accumulation of the vascular tracer protein, ^{131}I-albumin, into the air spaces of the lungs, the concentration of ^{131}I-albumin in the alveolar sample was measured at the end of the experiment and compared with the concentration in the plasma.

Alveolar Fluid Clearance. Changes in the concentration of the instilled nonlabeled bovine albumin over the study period (1 h) were used to measure fluid clearance from the distal air spaces, as we have in several previous experimental studies.[2,8] An increase in alveolar protein concentration is a direct measurement of absorption of water from the instilled solution since very little protein entered or escaped from the alveolar space during the studies (see Results). Because some reabsorption may have occurred across distal bronchial epithelium, the term *alveolar* does not imply that all fluid reabsorption occurred at the alveolar level.

Tracer Binding Measurement. To determine ^{125}I binding to albumin, precipitation of all protein in the samples was performed by adding 20% trichloroacetic acid to all tubes. The tubes were then centrifuged to obtain the supernatant for measurement of free ^{125}I radioactivity. The results are expressed as a percentage of the unbound ^{125}I radioactivity to the total amount of ^{125}I-albumin radioactivity instilled. All fluid samples always had less than 1% of unbound activity.

In Vitro *Studies*

To further test the hypothesis that lidocaine acts on the basolateral surface of the alveolar epithelium, we evaluated the effect of lidocaine on the ENaC, a channel that is expressed only on the apical surface of alveolar epithelial cells.[21] To test the hypothesis that lidocaine did not act on ENaC, the following studies were conducted. Because oocyte expression studies suggest that all three subunits (α, β, γ) are required for optimal function of the channel,[22] *Xenopus* oocytes were transfected with all three human subunits of ENaC.

Oocytes Injected with Apical Epithelial Sodium Channel. Mature female *Xenopus laevis* were maintained at 20°C with a 12-h light–dark cycle. Individual females were anesthetized in ice, and oocyte clusters were surgically removed from the ovary. Oocyte clusters were torn apart with forceps in ND96 medium containing 96 mM NaCl, 2 mM KCl, 10 mM HEPES, and 1.8 mM $CaCl_2$ at pH 7.4. Denuded oocytes were obtained by collagenase digestion (type IA, Sigma, 370 U/ml) during 2 h at room temperature and rinsed several times in ND96. Stage 5–6 oocytes were selected and incubated overnight at 18°C in ND96 medium with gentamycin (50 mg/ml). Healthy oocytes were then selected and injected with 50 nl cRNA (50 ng/ml). The oocytes were incubated for 1 day after the injection in ND96 medium supplemented with 50 mg/ml gentamycin, 10 μM amiloride, and 5% inactivated horse serum. For the measurements in this study, the oocytes were punctured with two conventional microelectrodes (3 mol/1 KCl) for voltage clamp experiments (TEV200 voltage clamp; Dagan Corporation, Minneapolis, MN). The specific ENaC current was defined as the total current recorded at -70 mV holding potential minus the current recorded at the same holding potential in the presence of 20 μM amiloride. Recordings were performed in ND96, where $CaCl_2$ was replaced by 1.8 mM $BaCl_2$ to block endogenous oocyte K^+ conductance.

Statistics

All data are summarized as mean $\pm$ SE. Experimental and control groups were compared using one-way analysis of variance if significant by *post hoc* Sheffé test. $P < 0.05$ was considered statistically significant.

Results

Hemodynamics, Airway Pressure, and Arterial Blood Gases

The mean systemic arterial pressure, peak airway pressure, and arterial blood gases were similar at baseline in all groups. There was no effect of intraalveolar or intravenous lidocaine on mean systemic arterial pressure or oxygenation in rats (table 1).

Table 1. Mean Systemic Arterial Pressure and Pao_2/Fio_2 Ratio 30 Minutes After Starting Lidocaine IV Administration in Lidocaine IV Group

Experimental Conditions	n	Systemic Arterial Pressure (mmHg)	PaO_2/FIO_2
Control	6	92 ± 7	530 ± 58
Lidocaine IV	6	94 ± 4	545 ± 96
Lidocaine alv	5	95 ± 9	528 ± 76
Amiloride alv	4	90 ± 6	565 ± 85
Lidocaine alv + amiloride alv	4	100 ± 10	502 ± 24
Terbutaline	4	100 ± 11	482 ± 85
Lidocaine alv + terbutaline alv	4	100 ± 13	479 ± 38

Values are mean ± SE in mmHg.

* $P < 0.05$ *vs.* controls.

n = number of rats studied; alv = in alveoli; IV = intravenous.

Effects of Lidocaine on Alveolar Fluid Clearance

Alveolar lidocaine (10^{-5} M) decreased alveolar fluid clearance over 1 h by 50% compared with control rats (fig. 1). There were similar effects with intravenous lidocaine (plasma concentration, 10^{-5} M) on alveolar fluid clearance over 1 h (fig. 1).

Effects of β-adrenergic Stimulation on the Lidocaine-induced Decrease in Alveolar Fluid Clearance

The β-adrenergic agonist, terbutaline, increased alveolar fluid clearance in control rats by 30% (fig. 2). Interestingly, terbutaline overcame the effect of lidocaine, resulting in the normal up-regulation of alveolar fluid clearance with β_2-agonist stimulation (fig. 2).
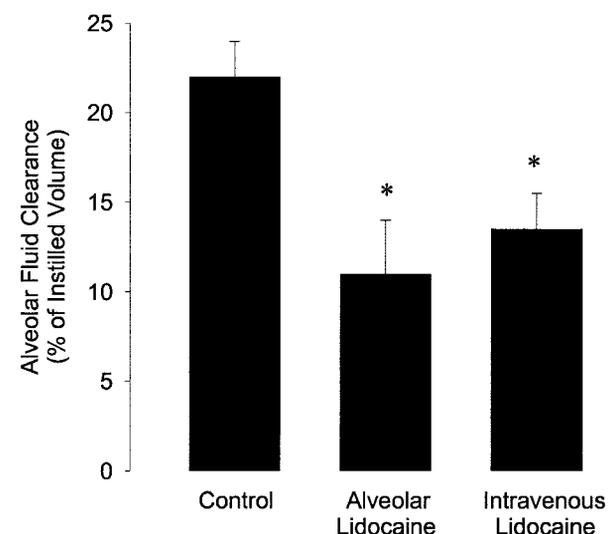

Fig. 1. Alveolar fluid clearance (% instilled) over 1 h, after intraalveolar lidocaine (10^{-5} M; n = 5) or intravenous lidocaine (2 mg/kg + 2 mg · kg^{-1} · h^{-1}; n = 6) with the same range of plasma concentration. Alveolar fluid clearance was decreased in both groups after lidocaine compared with control rats (n = 6). Values are mean ± SE. *$P < 0.05$ *versus* controls.
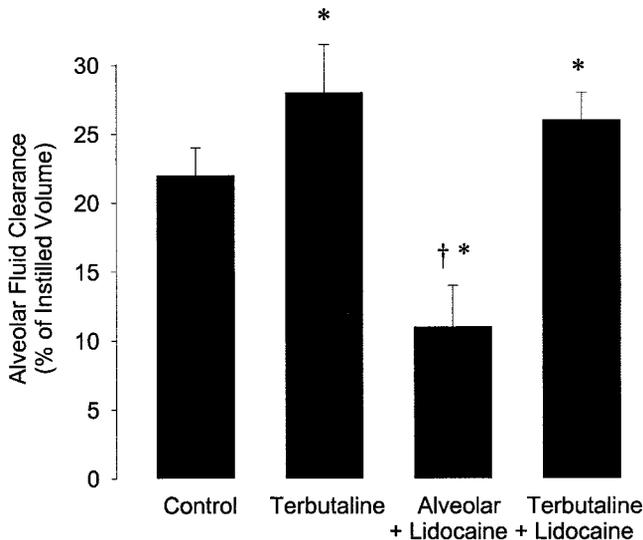
Fig. 2. Effect of alveolar terbutaline (10^{-4} M; n = 4) on alveolar fluid clearance over 1 h with or without lidocaine (10^{-5} M; n = 4). Terbutaline increased alveolar fluid clearance compared with both control (n = 6) and lidocaine-treated rats. Values are mean ± SE. *P < 0.05 *versus* controls; †P < 0.05 *versus* terbutaline.

Effects of Lidocaine on Amiloride Inhibition of Alveolar Fluid Clearance

Amiloride (10^{-4} M) inhibited alveolar fluid clearance in control rats by approximately 60%. When amiloride was added to the instilled albumin solution with lidocaine, there was no additional inhibition of alveolar fluid clearance (fig. 3).

Effect of n-Ethyl Bromide Quaternary Salt on Alveolar Fluid Clearance

When QX-314 (10^{-5} M) was added to the instilled albumin solution, there was no effect, similar to the
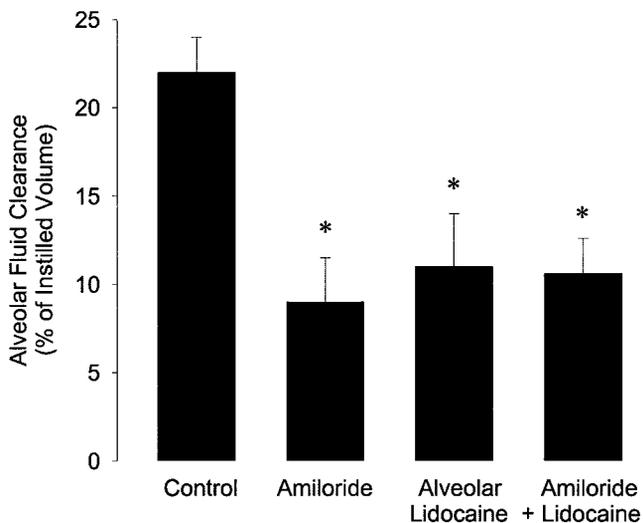

Fig. 3. Effect of amiloride (10^{-3} M) on alveolar fluid clearance (% instilled) over 1 h with (n = 4) or without (n = 4) lidocaine (10^{-5} M). Amiloride reduced alveolar fluid clearance over 1 h compared with control (n = 5); there was no additive effect with lidocaine. Values are mean ± SE. *P < 0.05 *versus* controls.
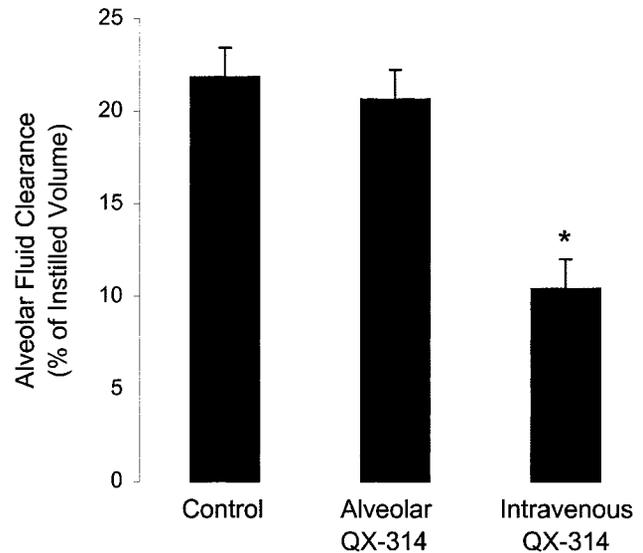
Fig. 4. Effect of the positively charged lidocaine analog QX-314 instilled either in the alveolar space (10^{-5} M; n = 3) or intravenously (n = 3) at the same dose as intravenous lidocaine, *i.e.*, a bolus 2 mg/kg dose followed by a continuous infusion at a rate of 2 mg · kg^{-1} · h^{-1} until the end of experiment (n = 3). Intravenous QX-314 decreased alveolar liquid clearance over 1 h compared with controls; there was no effect when QX-314 was given in the air spaces. Values are mean ± SE. *P < 0.05 *versus* controls.

control studies. By contrast, intravenous QX-314 decreased alveolar fluid clearance over 1 h by 50%, similar to the effect of intravenous lidocaine (fig. 4).

Protein Movement across the Endothelial and Epithelial Barriers of the Lung

There was no effect of intravenous or intraalveolar lidocaine on either the movement of the vascular tracer (^{131}I-albumin) into the air spaces of the lung or the alveolar tracer (^{125}I-albumin) from the lung to the plasma compared with the controls. Thus, there was no change in epithelial or endothelial permeability to protein in the lung (table 2).

Effect of Lidocaine on Apical Epithelial Sodium Channel Subunits in Xenopus Oocytes

Lidocaine had no direct effects on the ENaC. This result was demonstrated after heterologous expression of the three human ENaC subunits in *Xenopus* oocytes. A large amiloride-sensitive current developed in the oocytes 1 day after injection, and this current was not altered by 10 μM lidocaine (fig. 5).

Discussion

There were several clinical reasons for studying the effect of lidocaine on alveolar epithelial fluid clearance in the lung. First, lidocaine has been reported to impair ion transport in nasal and tracheal epithelium.[10,11] If this effect occurred at the level of the alveolar or distal

Table 2. Effect of IV or Intraalveolar Lidocaine on Protein Permeability across the Lung Epithelial Barrier Assessed by Accumulation of an Alveolar Protein Tracer (^{125}I-albumin) in the Plasma and the Alveolar/Plasma Ratio of a Vascular Protein Tracer (^{131}I-albumin)

Experimental Conditions	n	Alveolar Protein Tracer ^{125}I-albumin (% of instilled)		^{131}I-albumin Alveolar/Plasma Ratio
		Lung	Plasma	
Control	6	99.7 ± 0.5	0.3 ± 0.3	0.07 ± 0.02
Lidocaine alv	5	99.7 ± 0.3	0.2 ± 0.2	0.11 ± 0.06
Lidocaine IV	6	99.7 ± 0.3	0.2 ± 0.2	0.06 ± 0.08

Values are means ± SE.

* $P > 0.05$ *vs.* control.

n = number of rats studied; IV = intravenous; alv = in alveoli.

airway epithelium, then lidocaine could either lower the threshold for developing alveolar edema or slow the resolution of edema because active ion transport is the primary mechanism responsible for the resolution of alveolar edema.[2,3,6,8] Interestingly, recent clinical studies have implicated lidocaine as a possible cause of pulmonary edema in patients undergoing liposuction surgery.[17,18,23] In addition, because lidocaine is widely used as an antiarrhythmic agent in patients with cardiac disease who are susceptible to the development of pulmo-
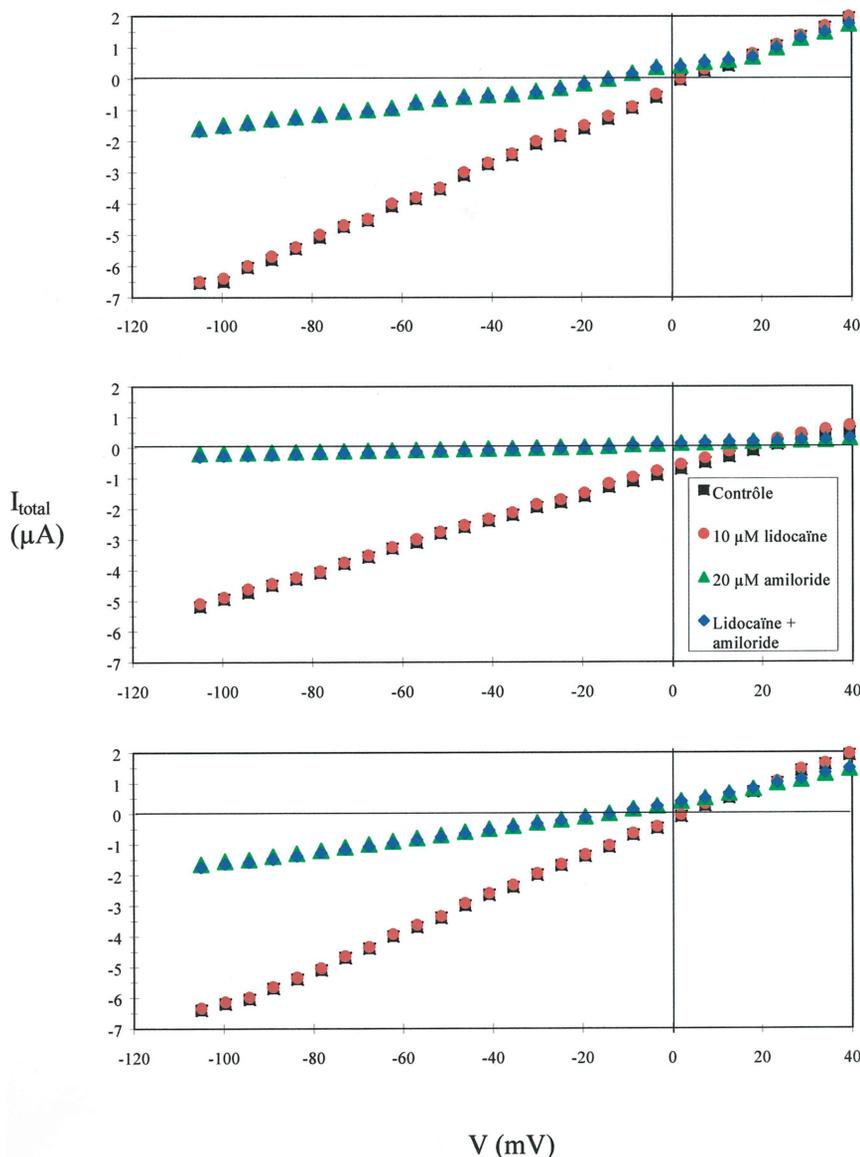

Fig. 5. Current–voltage relation of the amiloride-sensitive Na$^+$ channel (all three subunits of ENaC) when expressed in *Xenopus* oocytes. Lidocaine (10 μM) was added in the presence or absence of amiloride (20 μM). The figure shows the results of three separate experiments. In all of the studies, the control and lidocaine current overlapped, as well as the amiloride and amiloride–lidocaine current. Thus, there was no effect of lidocaine. In addition, there was no effect of lidocaine alone (data not shown). Similar results were obtained with two different batches of oocytes.

I_{total} (μA)

V (mV)

Contrôle
10 μM lidocaïne
20 μM amiloride
Lidocaïne + amiloride

nary edema, it is important to know if lidocaine can impair alveolar epithelial fluid transport. Also, the post-operative use of lidocaine in patients with acute cardiac disease could lower the threshold for developing pulmonary edema.

The principal results of this study can be summarized as follows. Lidocaine decreased baseline alveolar epithelial fluid clearance by 50%. This effect occurred whether lidocaine was administered intravenously in a clinically relevant dose or directly in the distal airspaces of the lung. β-adrenergic stimulation with terbutaline was successful in overcoming the lidocaine effect and restoring alveolar fluid clearance to the normal up-regulated level.[2,6] There may be several mechanisms by which lidocaine could inhibit alveolar epithelial fluid clearance.

First, does lidocaine act directly on transepithelial sodium transport? It is well accepted that alveolar fluid clearance is driven, in part, by amiloride-sensitive and -insensitive sodium transport.[6,21] Because sodium transport is an important driving force, and lidocaine can induce a blockade of voltage-gated channels through interference with the conformational changes that underlie the activation process,[12] it is possible that inhibition of sodium channels would be one mechanism. There are sodium channels present in the alveolar epithelium that may be candidates for inhibition by lidocaine. The most likely candidate on the apical surface would be the ENaC. However, when lidocaine was instilled into the lungs together with amiloride, no additional inhibition occurred. This result suggested that lidocaine and amiloride might compete for the same inhibitory site on ENaC. A second possibility would be that lidocaine inhibited an accessory sodium transport pathway that required amiloride inhibition, a theory that could explain why amiloride did not induce a further decrease of alveolar liquid clearance in the presence of lidocaine.

Therefore, experiments to investigate the first hypothesis were conducted. First, we evaluated the effect of lidocaine on the function of ENaC expressed in *Xenopus* oocytes. In these studies, lidocaine had no direct effects on the function of ENaC. To investigate the second hypothesis, we used a permanent polarized analog of lidocaine, QX-314, and administered this compound either intravenously or into the distal air spaces of the lung. The decrease in alveolar fluid clearance occurred only with intravenous administration of QX-314 and not when QX-314 was administered into the distal airspaces of the lung. This result supports the hypothesis that the primary effect of lidocaine occurs from its action on the basal side of the alveolar epithelium. Thus, the lidocaine-induced decrease in alveolar fluid clearance apparently was not the result of a decrease in sodium channel apical uptake since intraalveolar QX-314 did not decrease alveolar fluid clearance and because there was no direct effect of lidocaine on ENaC activity in the oocytes. Thus,

the effect of lidocaine must be explained by another mechanism.

Could lidocaine exert its effect by inhibiting the basolateral NaKATPase or potassium channels, or by both mechanisms? Because QX-314 was able to reduce the alveolar fluid clearance intravenously but not in alveolar spaces, these data suggested that lidocaine acts primarily on the basal side of the alveolar epithelium. This inhibition could have been the result of either a direct effect on the NaKATPase activity or a secondary inhibition of potassium channels. Theoretically, lidocaine may have altered the activity of NaKATPase, although there is no previous report that lidocaine has this effect. On the other hand, it is well known that lidocaine can inhibit basolateral K^+ channels in renal brush-border membranes,[24] urinary bladder epithelia,[25,26] turtle colon epithelial cells,[27] and frog alveolar epithelium.[14] Our studies do not resolve whether the effect of lidocaine in these *in vivo* studies is mediated by a reduction in the activity of NaKATPase or inhibition of K^+ channels, or both. However, we favor the latter explanation, that lidocaine inhibits basal potassium channels, because of previous *in vitro* work demonstrating this effect.[14,28] In addition, a previously published study from our research group demonstrated that a potassium channel opener (YM934) increased the rate of alveolar fluid clearance in an *ex vivo* human lung preparation.[29] Thus, in the current study, lidocaine may well have had the opposite effect on potassium channels.

How would these mechanisms explain the lidocaine-induced decrease in vectorial salt and water transport across the alveolar epithelium? In tight epithelia there is a relation between ion transport of the apical membrane sodium-selective uptake and the basolateral membrane potassium-selective extrusion. Normally, an increase of sodium uptake across the apical membrane leads to an increased extrusion of sodium at the basolateral membrane by the NaKATPase to maintain a constant low intracellular sodium concentration. This effect is associated with an increase in basal membrane conductance of potassium channels. In urinary bladder, inhibition of apical sodium channels by amiloride results in a decrease in basolateral membrane potassium permeability. Conversely, the blockade of basolateral potassium channels leads to a decrease in apical sodium conductance.[30]

Why was terbutaline able to reverse most of the inhibitory effect of lidocaine? It is well known that β-adrenergic agonists, such as terbutaline or isoproterenol, can increase the activity of NaKATPase in alveolar type II cells.[31,32] NaKATPase activity enhances extrusion of sodium, resulting in enhanced apical sodium uptake. To maintain the cell potential, potassium needs to be extruded by basolateral potassium channels. In the presence of lidocaine, this extrusion is still possible because different subtypes of basolateral potassium channels exist and only some of them are inhibited by lidocaine.[33]

The blockade of all basolateral potassium channels would antagonize the stimulatory effect of terbutaline on alveolar fluid transport similar to the effect reported in fetal distal lung epithelium *in vitro* with barium, a non-specific potassium channel blocker.[34]

What is the clinical significance of these results? Whatever the mechanisms responsible for the formation of pulmonary edema, a decrease in the alveolar epithelial fluid transport capacity can delay or impair the resolution of alveolar edema. We found that in critically ill patients ventilated for acute respiratory distress syndrome, the patients who were able to maximize alveolar fluid clearance had a better outcome.[35,36] Critically ill patients with cardiac arrhythmias receive the same dose of lidocaine intravenously ($2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) that was administered in this experimental study. Many of these patients are predisposed to develop hydrostatic pulmonary edema because of acute and chronic cardiac disease. Conceivably, lidocaine therapy may lower the threshold for the development of alveolar edema, especially in the presence of elevated left atrial pressure. In addition, there have been several reports of pulmonary edema that occurred after plastic surgery, especially liposuction, concomitant with the use of high doses of lidocaine along with fluid loading.[16,18] In the presence of volume loading and a lidocaine-induced reduction in cardiac contractility, inhibition of alveolar epithelial fluid transport could contribute to or worsen the development of pulmonary edema.

In summary, clinically relevant concentrations of lidocaine reduced alveolar epithelial fluid transport in rats. The inhibitory effect of lidocaine was probably mediated by inhibition of potassium channels or NaKATPase activity on alveolar epithelium. The inhibitory effect of lidocaine was overcome with β-adrenergic receptor stimulation. This reversibility may be particularly relevant to patients with cardiac failure who are often treated with dobutamine, an agent that can augment alveolar fluid clearance in rats by stimulation of β_2-adrenergic receptors.[37]

References

1. Börjesson A, Norlin A, Wang X, Anderson R, Folkesson HG: TNFα stimulates alveolar liquid clearance during intestinal ischemia reperfusion in rats. Am J Physiol 2000; 278:L3–12

2. Pittet J-F, Wiener-Kronish JP, McElroy MC, Folkesson HG, Matthay MA: Stimulation of lung epithelial liquid clearance by endogenous release of catecholamines in septic shock in anesthetized rats. J Clin Invest 1994; 94:663–71

3. Rezaiguia S, Garat C, Delclaux C, Meignan M, Fleury J, Legrand P, Matthay MA, Jayr C: Acute bacterial pneumonia in rats increases alveolar epithelial fluid clearance by a tumor necrosis factor-alpha-dependent mechanism. J Clin Invest 1997; 99:325–35

4. Verghese GM, Ware LB, Matthay BA, Matthay MA: Alveolar epithelial fluid transport and resolution of clinical severe hydrostatic pulmonary edema. J Appl Physiol 1999; 87:1301–12

5. Ware LB, Golden JA, Finkbeiner WE, Matthay MA: Alveolar epithelial fluid transport capacity in reperfusion lung injury after lung transplantation. Am J Respir Crit Care Med 1999; 159:980–8

6. Matthay MA, Folkesson HG, Verkman AS: Salt and water transport across alveolar and distal airway in adult lung. Am J Physiol 1996; 270:L487–503

7. Minakata Y, Suzuki S, Gryorczyk C, Dagenais A, Berthiaume Y: Impact of beta-adrenergic agonist on Na+-K+-ATPase expression in alveolar type II cells. Am J Physiol 1998; 275:L414–22

8. Rezaiguia-Delclaux S, Jayr C, Luo DF, Saïdi N-E, Meignan M, Duvaldestin P: Halothane and isoflurane decrease alveolar epithelial fluid clearance in rats. ANESTHESIOLOGY 1998; 88:751–60

9. Molliex S, Dureuil B, Aubier M, Friedlander G, Desmonts J-M, Clerici C: Halothane decreases Na, K-ATPase, and Na channel activity in alveolar type II cells. ANESTHESIOLOGY 1998; 88:1606–13

10. Knowles MR, Carson JL, Collier AM, Gatzy JT, Boucher RC: Measurements of nasal transepithelial electric potential differences in normal human subjects in vivo. Am Rev Respir Dis 1981; 124:484–90

11. Knowles RM, Buntin WH, Bromberg PA, Gatzy JT, Boucher RC: Measurements of transepithelial electric potential differences in trachea and bronchi of human subjects in vivo. Am Rev Respir Dis 1982; 126:108–12

12. Butterworth JF, Strichartz GR: Molecular mechanisms of local anesthesia: A review. ANESTHESIOLOGY 1990; 72:711–34

13. Lee-Son S, Wang GK, Concus A, Crill E, Strichartz G: Stereoselective inhibition of neuronal sodium channels by local anesthetics. ANESTHESIOLOGY 1992; 77:324–35

14. Illek B, Fisher H, Clauss W: Aldosterone regulation of basolateral potassium channels in alveolar epithelium. Am J Physiol 1990; 259:L230–7

15. Dawson DC, Richards NW: Basolateral K conductance: Role in regulation of NaCl absorption and secretion. Am J Physiol 1990; 259:C181–95

16. Gilliland MD, Coates N: Tumescent liposuction complicated by pulmonary edema. Plast Reconstr Surg 1997; 99:215–9

17. Pitman GH: Tumescent liposuction complicated by pulmonary edema. Plast Reconstr Surg 1997; 100:1363–4

18. Rao RB, Ely SF, Hoffman RS: Deaths related to liposuction. N Engl J Med 1999; 340:1471–5

19. Nishima K, Mikawa K, Takao Y, Shiga M, Maekawa N, Obara H: Intravenous lidocaine attenuates acute lung injury induced by hypochloric aspiration in rabbits. ANESTHESIOLOGY 1998; 88:1300–9

20. Matthay MA, Berthiaume Y, Staub NC: Long-term clearance of liquid and protein from the lungs of unanesthetized sheep. J Appl Physiol 1985; 59:928–34

21. Matalon S, Benos DJ, Jackson RM: Biophysical and molecular properties of amiloride-inhibitable Na+ channels in alveolar epithelial cells. Am J Physiol 1996; 271:L1–22

22. Barbry P, Hoffman P: Molecular biology of Na+ absorption. Am J Physiol 1997; 273:G571–85

23. Ostad A, Kageyama N, Moy RL: Tumescent anesthesia with a lidocaine dose of 55 mg/kg is safe for liposuction. Dermatol Surg 1996; 22:921–7

24. Schell RE, Wright EM: Effects of lidocaine on transport properties of renal brush-border membranes. Biochim Biophys Acta 1987; 896:256–62

25. Van Driessche W: Lidocaine blockage of basolateral potassium channels in the amphibian urinary bladder. J Physiol (Lond) 1986; 381:575–93

26. Van Driessche W, Erlij D: Activation of K+ conductance in basolateral membrane of toad urinary bladder by oxytocin and cAMP. Am J Physiol 1988; 254:C816–21

27. Richards NW, Dawson DC: Single potassium channels blocked by lidocaine and quinidine in isolated turtle colon epithelial cells. Am J Physiol 1986; 251:C85–6

28. Sakuta H, Sekiguchi M, Okamoto K, Sakai Y: Inactivation of gilbenclamide-sensitive K+ channels in Xenopus oocyte by various calmodulin antagonists. Eur J Pharmacol 1992; 226:199–207

29. Sakuma T, Takahashi K, Ohya N, Matthay MA: Effects of ATP-sensitive potassium channel opener on potassium transport and alveolar fluid clearance in the resected human lung. Pharmacol Toxicol 1998; 83:16–22

30. Demarest JR, Finn A: Interaction between the basolateral K+ and apical Na+ conductance in Necturus urinary bladder. J Gen Physiol 1987; 89:563–80

31. Campbell AR, Folkesson HG, Berthiaume Y, Gutkowska J, Matthay MA: Alveolar epithelial fluid clearance persists in the presence of moderate left atrial hypertension in sheep. J Appl Physiol 1999; 86:139–51

32. Minakata Y, Suzuki S, Grygorczyk C, Dagenais A, Berthiaume Y: Impact of beta-adrenergic agonist on Na+ channel and Na+-K+-ATPase expression in alveolar type II cells. Am J Physiol 1998; 275:L414–22

33. DeCoursey TE, Jacobs ER, Silver MR: Potassium currents in rat type II alveolar epithelial cells. J Physiol (Lond) 1988; 395:487–505

34. O'Brodovich H, Rafii B: Effect of K channel blockers on basal and β-agonist stimulated ion transport by fetal distal lung epithelium. Can J Physiol Pharmacol 1993; 71:54–7

35. Matthay MA, Wiener-Kronish JP: Intact epithelial barrier function is critical for the resolution of alveolar edema in humans. Am Rev Respir Dis 1990; 142:1250–7

36. Ware LB, Matthay MA: Alveolar fluid clearance is impaired in the majority of patient with acute lung injury and the acute respiratory distress syndrome. Am J Respir Crit Care Med 2001; 163:1376–83

37. Tibayan FA, Chesnutt AN, Folkesson HG, Eandi J, Mattay MA: Dobutamine increases alveolar liquid clearance in ventilated rats by beta-2 receptors stimulation. Am J Respir Crit Care Med 1997; 156:438–44