Local Anesthetic Contracture and Relaxation of Airway Smooth Muscle

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Recent studies have suggested that local anesthetics administered as an aerosol may have clinical use as bronchodilators. To assess direct actions on airway smooth muscle the authors studied the effects of five local anesthetics on intrinsic and agonist-induced tone of guinea pig tracheal chains. Drug effects were recorded with an isotonic transducer against a tone of either 300 mg or 4 g and were expressed as percentages of the maximal relaxation produced by isoproterenol (10⁻⁴ M) or the maximal contracture produced by acetylcholine (10⁻⁴ M). Bath solution was usually maintained at pH 7.5, but the effects of lidocaine were also studied at pH 7.0 and pH 8.0, produced by changing the concentrations of carbon dioxide and bicarbonate ion. In lightly weighted tracheal chains, tetracaine produced purely relaxant effects, whereas lidocaine, bupivacaine, procaine, and tetracaine produced contracture at low concentrations and relaxation at higher concentrations. The transition from contracture to relaxation occurred at a breakpoint concentration characteristic for each anesthetic. Lidocaine produced dose-related contracture from 3 × 10⁻⁵ to 10⁻⁴ M, with greater effect (P < .05) at pH 7.0 than at pH 8.0; correlation coefficients for contracture as an exponential function of lidocaine concentration were significantly greater (P < .01) for ionized drug (r = 0.96) or total drug (r = 0.92) than for nonionized drug (r = 0.71). All local anesthetics produced predominantly relaxant effects when tested in heavily weighted chains that were partially contracted with carbamylcholine (3 × 10⁻⁷ M) or histamine (3 × 10⁻⁵ M). At low concentrations, 3 × 10⁻⁵ to 10⁻⁴ M, procaine (P < .001), tetracaine (P < .05) and bupivacaine (P < .05) were more effective against carbamylcholine than histamine; the low-dose anticholinergic effects of procaine and tetracaine were greater (P < .01) than those of other local anesthetics and at 10⁻⁴ M produced relaxation equivalent to 47 ± 3 (±SE) and 40 ± 7 per cent, respectively, of the maximal effect of isoproterenol. At high concentration, 10⁻³ M, procaine, bupivacaine, and tetracaine produced nearly complete relaxation of both carbamylcholine- and histamine-enhanced tone, but the relaxant effects of lidocaine and procaine were significantly less (P < .01). Our results show that there are marked differences in the direct effects of local anesthetics on airway smooth muscle that could affect their clinical use as bronchodilators. These differences include extent of contracture, anticholinergic effects at low concentrations, and nonselective relaxant effects at high concentration. The poor correlation of contracture with concentration of nonionized drug suggests a superficial site of action for the contractor effect. (Key words: Anesthetics, local, bupivacaine; Anesthetics, local, lidocaine; Anesthetics, local, procaine; Anesthetics, local, tetracaine; Lung, trachea; Airway, muscle tone, dilatation; Histamine.)

Severe bronchospasm occurring during general anesthesia is a rare but potentially lethal anesthetic emergency. The most common treatment has been to deepen the level of anesthesia, usually by the administration of halothane. Because of the risk of ventricular dysrhythmias, beta-adrenergic stimulants are usually avoided. Systemic administration of local anesthetics is an alternative therapy that has been used to treat bronchospasm, including that occurring during general anesthesia. Recent studies in animals have shown that local anesthetics administered as an aerosol are also effective against experimentally evoked bronchospasm. The efficacy of aerosol therapy may be due to depression of irritant receptors in the lower airway, block of central nervous system reflex responses after systemic absorption, block of vagal efferent activity, or direct depression of airway smooth muscle. To assess direct actions on airway smooth muscle that might contribute to, or detract from, bronchodilation, we studied the effects of local anesthetics on intrinsic and agonist-induced tone of guinea pig tracheal chains. Five local anesthetics were compared, and lidocaine was studied at three values of pH to evaluate the relative effects of ionized and nonionized drug.

Methods

Male guinean pigs weighing between 400 and 800 g were sacrificed by a blow on the head and the tracheas removed from larynx to carina. Tracheal chains were prepared as described by Castillo and deBeer, as modified by a pairing procedure similar to that of Foster. In the pairing procedure the tracheas were removed from two guinea pigs and rings, 2–3 mm wide, were cut from each trachea, beginning at the laryngeal end. The rings were cut alternately from each trachea and placed successively in two or three petri dishes containing Krebs-Henseleit solution. These were used to assemble two ("twin") or three ("triplicate") matched chains, each of eight rings in length and composed of pooled rings from both animals.

The rings of each chain were tied together with...
Fig. 1. Responses of lightly weighted tracheal chains to cumulative increases in concentration of lidocaine, tricaine and bupivacaine. Addition of drug is indicated by an arrow, with the cumulative drug concentration (m) shown below it. The responses to lidocaine and tricaine shown in A were obtained in twin chains. Both the contraction elicited by lidocaine and the relaxation elicited by tricaine were rapidly reversible when the bath solution was changed as indicated by W (wash). Trace B shows the contracture elicited by bupivacaine and the break point at \( 6 \times 10^{-4} \) m.

5-0 silk and mounted in a 50 ml organ bath containing Krebs-Henseleit solution maintained at 37.5°C. The Krebs-Henseleit solution contained the following chemicals in mmol/l concentrations: sodium chloride, 118; potassium chloride, 4.7; calcium chloride, 2.5; magnesium sulfate, 0.6; monobasic potassium phosphate, 1.2; sodium bicarbonate, 25; glucose, 11.1. The bath solution was aerated through a sintered glass disc at the bottom of each chamber and pH of the bath solution was monitored in a separate chamber of identical construction using a Beckman 76008 pH meter and 39183 probe electrode. In most experiments the bath solution was aerated with a mixture of carbon dioxide, 5 per cent, and oxygen, 95 per cent, which produced a pH of 7.5 (7.46–7.56). To study drug effect at pH 8.0 (7.96–8.04) the carbon dioxide concentration was decreased to 1.38 per cent; for pH 7.0 (6.96–7.03) the carbon dioxide concentration was increased to 7.03 per cent and the bicarbonate concentration was decreased to 12.5 mmol/l. A \( pK_a \) of 7.86\textsuperscript{11,12} was used to calculate the concentrations of ionized and nonionized lidocaine at each pH.

Contractions were recorded isotonically with a transducer (Harvard 356) at a gain of about 15X and against a tension of either 500 mg ("lightly weighted") or 4 g ("heavily weighted"). Before testing drug effect, the chains were allowed to equilibrate for at least an hour until a stable resting length had been reached. To determine cumulative dose-response relationships the local anesthetics were added in progressively increasing concentrations without washout between drug additions. Sufficient time was allowed to obtain the maximal effect of each concentration. In lightly weighted chains the local anesthetics were tested for effect on intrinsic tone. In heavily weighted chains they were tested first against tone enhanced by carbamylcholine (\( 3 \times 10^{-7} \) m) and then against tone enhanced by histamine (\( 3 \times 10^{-6} \) m). At the end of each experiment the preparations were maximally relaxed with increasing concentrations of isoproterenol (\( 10^{-9}, 10^{-8}, 10^{-7} \) and \( 10^{-6} \) m) and maximally contracted with increased concentrations of acetylcholine (\( 10^{-6}, 10^{-5}, 10^{-4} \) and \( 10^{-3} \) m). The effects of the local anesthetics were expressed as percentages of the maximal relaxation produced by isoproterenol or the maximal contracture elicited by acetylcholine. Twin chains were used to compare the effects of tricaine and lidocaine (Group I), and twin or triplicate chains obtained from other animals were used to test the effects of bupivacaine, tetracaine, and procaine. Triplicate chains were also used to test the effects of lidocaine (Group II) at different values of pH; one chain from each set was tested at pH 7.0, 7.5, and 8.0.

Acetylcholine chloride, carbamylcholine chloride, histamine dihydrochloride, \( d,l \)-isoproterenol hydrochloride, tetracaine hydrochloride, procaine hydrochloride, bupivacaine hydrochloride monohydrate, lidocaine hydrochloride monohydrate and tricaine methanesulfonate were dissolved in 0.9 per cent sodium chloride solution and added to the organ.
baths in increments of 0.5 ml or less. Drug concentrations refer to concentrations in the organ baths. Against agonist-enhanced tone, the local anesthetics were tested at 3 × 10^{-5}, 10^{-4}, 3 × 10^{-4}, and 10^{-3} M. Against intrinsic tone the concentrations employed were as follows: bupivacaine and tetracaine, 10^{-5}, 10^{-4}, 3 × 10^{-4}, 6 × 10^{-4} and 10^{-3} m; procaine, 10^{-3}, 10^{-4}, 3 × 10^{-4}, 10^{-3}, 3 × 10^{-3}, 6 × 10^{-3} and 10^{-2} m; lidocaine (Group I) and tetracaine, 10^{-3}, 3 × 10^{-3}, 10^{-4}, 3 × 10^{-4} and 10^{-3} m; lidocaine (Group II), 10^{-3}, 3 × 10^{-3}, 10^{-4}, 3 × 10^{-4}, 10^{-3}, 3 × 10^{-3}, and 6 × 10^{-3} m.

**Results**

In lightly weighted tracheal chains, lidocaine, bupivacaine and tetracaine produced no consistent effect at 10^{-3} M and produced contracture at 10^{-4} M (fig. 1). At 3 × 10^{-4} M procaine also produced contracture. As drug concentration was increased, relaxation occurred abruptly as a "breakpoint" (fig. 1B) at a concentration characteristic for each anesthetic (table 1). Bupivacaine and tetracaine produced breakpoints at concentrations of less than 10^{-3} M, and as a consequence these anesthetics elicited less intense contractures (table 1). Lidocaine and procaine produced dose-related contractures until breakpoints at 3 × 10^{-3} and 10^{-2} M and elicited contractures equivalent to half of the maximal response to acetylcholine. A two- or threefold increase in drug concentration above the breakpoint produced near maximal relaxation. High concentrations of procaine, at or just below the breakpoint, caused irregular oscillations; these were not seen with any other local anesthetic. Tricaine did not elicit contracture (fig. 1A) and produced dose-related relaxation with maximal effect at 10^{-3} M (fig. 2).

The extent of contracture elicited by lidocaine in the concentration range from 3 × 10^{-3} to 10^{-3} M was a linear function of the logarithm of drug concentration at all values of experimental pH (fig. 3).

**Table 1. Breakpoint Concentrations* and Maximal Contractures†**

<table>
<thead>
<tr>
<th></th>
<th>Fraction of Chains Tested Responding with Relaxation at Drug Concentrations (n) of:</th>
<th>Maximal Contracture, Mean ± SE</th>
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<tbody>
<tr>
<td></td>
<td>10^{-4} 5 × 10^{-4} 6 × 10^{-4} 10^{-3} 3 × 10^{-3} 6 × 10^{-3} 10^{-2}</td>
<td></td>
</tr>
<tr>
<td>Tetracaine</td>
<td>0/5     3/5 5/5</td>
<td>24.9 ± 1.7</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>0/5     1/5 5/5</td>
<td>9.5 ± 4.5</td>
</tr>
<tr>
<td>Lidocaine, Group I</td>
<td>0/5 0/5 0/5</td>
<td>0/5 0/5</td>
</tr>
<tr>
<td>Procaine</td>
<td>0/5     0/5 0/5</td>
<td>52.3 ± 9.2</td>
</tr>
<tr>
<td>Lidocaine, Group II</td>
<td>pH 8.0</td>
<td>0/4 0/4 0/4</td>
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<td>pH 7.5 0/4 0/4 0/4</td>
<td>0/4 0/4</td>
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<td>pH 7.0 0/4 0/4 0/4</td>
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* Wilcoxon rank sum tests indicate the following significant differences (P < .05) in breakpoint concentrations: Procaine > lidocaine > bupivacaine or tetracaine; lidocaine pH 7.0 > lidocaine pH 7.5 or 8.0.
† Maximal contractures elicited by local anesthetics as percentages of the maximal effect of acetylcholine. Analysis of variance with studentized range tests indicates the following significant differences: Procaine > bupivacaine (P < .01) or tetracaine (P < .05); lidocaine > bupivacaine (P < .05); lidocaine, pH 7.0 > lidocaine, pH 7.5 or 8.0 (P < .05).
Fig. 3. Cumulative dose–response relationships for lidocaine-induced contracture of lightly weighted tracheal chains at different values of bath pH. The contracture is standardized as percentage of the maximal response to acetylcholine (10^{-5}M). Each point represents the mean of four experiments and brackets give the SE. Solid circles, open triangles and open circles show contractures elicited at pH 7.0, 7.5, and 8.0, respectively; these contractures were obtained in triplicate chains derived from the same animals. Analysis of variance with studentized range tests indicates that contractures were greater ($P < .05$) at pH 7.0 than at pH 8.0 at all concentrations and that contractures were greater ($P < .05$) at pH 7.0 than at pH 8.0 at concentrations of $3 \times 10^{-5}$ and $10^{-4}$ M.

Lowering pH shifted the dose–response curve to the left, raised the breakpoint concentration, and increased the maximal contracture (fig. 3 and table 1). The concentrations needed to elicit contracture equivalent to 20 per cent of the maximal effect of acetylcholine (ED$_{20}$) were calculated from the regression lines for the linear portions of the dose–response curves and served as a measure of potency of lidocaine at each experimental pH (fig. 4). The ED$_{20}$'s for the total drug were significantly less ($P < .05$) at pH 7.0 than at pH 8.0. When effect was measured as a function of ionized or nonionized drug, the ED$_{20}$'s for ionized drug were nearly identical at all three values of experimental pH, but the ED$_{20}$'s for nonionized drug differed significantly at each pH (fig. 4). Combining results from experiments at pH 7.0, 7.5, and 8.0, the correlation coefficients for contracture as a function of drug concentration were 0.96 for ionized lidocaine, 0.92 for total lidocaine, and 0.71 for nonionized lidocaine. The correlation coefficients for ionized and total drug were not significantly different, but both were significantly greater ($P < .01$) than the correlation coefficient for nonionized drug.

When the tone of heavily weighted tracheal chains was enhanced by carbamylcholine or histamine, the local anesthetics produced predominantly relaxant effects. At low concentrations ($3 \times 10^{-5}$ and $10^{-4}$ M) all of the local anesthetics except tricaine produced greater relaxation of carbamylcholine- than of histamine-enhanced tone (figs. 2 and 5). The difference was significant for tetracaine, procaine and

Fig. 4. Variation of ED$_{20}$ with pH. ED$_{20}$'s were calculated from the regression lines for contracture as an exponential function of lidocaine concentration and show the estimated concentrations of lidocaine (total, ionized or nonionized) needed to produce a contracture equivalent to 20 per cent of the maximal effect of acetylcholine. Brackets give the standard error of the estimate. If only one form of lidocaine is active in eliciting contracture, the ED$_{20}$'s for the active form should be identical at all values of experimental pH. There are no significant differences between ED$_{20}$'s calculated for ionized drug; ED$_{20}$'s calculated for nonionized drug are greater at pH 8.0 than at pH 7.5 ($P < .05$) or 7.0 ($P < .01$) and greater at pH 7.5 than at pH 7.0 ($P < .01$); ED$_{20}$'s calculated for total drug are greater at pH 8.0 than at pH 7.0 ($P < .05$).
bupivacaine, but not for lidocaine (fig. 5). At low concentrations, $9 \times 10^{-5}$ and $10^{-4} \text{M}$, procaine and tetracaine were significantly more effective than other local anesthetics in relaxing carbamylcholine-enhanced tone ($P < .01$; analysis of variance with studentized range tests). Increasing the concentration from $10^{-4}$ to $3 \times 10^{-4} \text{M}$ produced a marked increase in the relaxant effects of tricaine, bupivacaine and tetracaine against both carbamylcholine- and histamine-enhanced tone, and at $10^{-3} \text{M}$ these drugs caused near-maximal relaxation (figs. 2 and 5). In contrast, increasing the concentration of lidocaine or procaine from $10^{-4}$ to $10^{-3} \text{M}$ produced only a slight increase in relaxation or a contracture. Such contractures were elicited at $10^{-3} \text{M}$ by lidocaine in one of five histamine-treated preparations and by procaine in three of five carbamylcholine-treated preparations. Thus, at high concentration, $10^{-3} \text{M}$, procaine and lidocaine produced significantly less relaxation than other local anesthetics ($P < .01$).

**Discussion**

Miller and Awe$^{23}$ have recently demonstrated that lidocaine aerosols administered to patients with obstructive pulmonary disease can produce small but significant increases in airway resistance. This could represent the *in-vivo* correlate of the contractures seen
during experiments with isolated tissues. Even if contracture is not apparent as an increase in airway resistance, the mechanisms responsible for contracture might severely restrict the extent of relaxation that could be achieved through other actions. Local anesthetic-induced contractures have been previously reported to occur in tracheal chains and in other types of smooth muscle. Local anesthetics also produce contracture of skeletal muscle, although the concentrations required are higher than those generally effective in smooth muscle. Tricaine was the only local anesthetic that did not elicit contracture of tracheal chains. Since tricaine (ethyl m-amino benzoate) is an isomer of benzoic acid and lacks the alkyl amine group characteristic of most local anesthetics, an alkyl amine group is probably essential to contractor effect. The tone of tracheal chains before addition of local anesthetic was also crucial in determining whether contracture or relaxation was the dominant drug effect. Local anesthetic-induced contracture of vascular smooth muscle is similarly tone-dependent and is obscured by high sympathetic tone in vivo or by prior partial contraction with norepinephrine or potassium in vitro.

Weiss et al. tested lidocaine in guinea pig tracheal chains against contractures induced by acetylcholine, histamine or potassium and found no selectivity against a particular agonist. In our experiments also, lidocaine showed no significant selectivity. In contrast, low concentrations of procaine and tetracaine were markedly more effective against carbamylcholine than against histamine (fig. 5). Previous studies by Sinha have shown selective anticholinergic effects of procaine in cat tracheal chains. The low-dose anticholinergic effects were not significantly different (fig. 5) for procaine and tetracaine, and were therefore independent of local anesthetic potency. Such effects may account for much of the success of intravenous procaine in treating bronchospasm.

The breakpoint concentration serves as an index of the nonselective relaxant activity of higher drug concentrations and was inversely related to local anesthetic potency (table 1). Concentrations that relaxed intrinsic tone produced a marked increase in relaxation of agonist-enhanced tone. This was evident as an inflection in the dose–response curves of tetracaine and bupivacaine (fig. 5) affecting both carbamylcholine- and histamine-enhanced tone. The concentrations of procaine and lidocaine that were tested against agonist-enhanced tone were too low to produce an inflection, and the slopes of their dose-response curves remained shallow from $8 \times 10^{-5}$ to $10^{-5}$ M (fig. 5).

Decreasing the pH of the bath solution from 8.0 to 7.0 increased the potency and maximal effect of lidocaine as a contractor of smooth muscle and increased the breakpoint concentration. The lower breakpoint at the more alkaline pH suggests that nonionized lidocaine was responsible for relaxation. This is consistent with previous work by Weiss et al. showing that the relaxant effect of lidocaine on histamine-enhanced tone in guinea pig tracheal chains was increased by increasing pH from 6.7 to 7.9. It is also consistent with the relaxant effect of tricaine ($pK_a = 3.5$), which is not ionized at physiologic pH. The increase in contractor potency at lower pH is subject to two alternative explanations, based on differences in effects of ionized and nonionized drug. The first assumes that only ionized lidocaine is active in eliciting contracture; the second, that both ionized and nonionized drug elicit contracture but that contracture is partially antagonized by the relaxant effects of nonionized drug at other sites of action. Our data cannot discriminate between these two explanations. In either case the contractor response should be, and is (fig. 4), closely tied to the concentration of ionized drug, which represents either the sole active form or total drug minus the concentration of the antagonist. Our results are opposite to those in skeletal muscle or vas deferens, in which local anesthetic-induced contractures are enhanced by alkaline pH. In skeletal muscle the contractor represents displacement of calcium from binding sites on the sarcoplasmic reticulum by nonionized drug, and in vas deferens the contractor results from release of endogenous catecholamines. Both mechanisms require passage of local anesthetic across cell membranes. Since only nonionized drug can readily cross biologic membranes, our data suggest that the site of action of local anesthetics in eliciting contracture of airway smooth muscle is on the surface of the cell membrane. Our data further suggest that systemic acidosis or acidic drug solutions might seriously impair bronchodilator effects by increasing the contractor response.

References

Obstetric Anesthesia

ROLL-OVER TEST A study of the capability of the roll-over test to predict pregnancy-induced hypertension was undertaken. Sixty primigravid and 60 multigravid patients were studied between the twenty-eighth and thirty-second weeks of gestation. The patients were chosen at random. All results were recorded but were not available to the physicians. Eighteen months later, after all study patients had delivered, the hospital charts and the patients' office records were evaluated to determine whether pregnancy-induced hypertension had occurred. In primigravid patients a positive test accurately predicted the later development of pregnancy-induced hypertension only 50 per cent of the time, while a negative test accurately predicted that it would not develop 98 per cent of the time. In multigravid patients, only 25 per cent of the patients who had positive tests later developed hypertension. The negative test in multigravid patients was accurate 94 per cent of the time. (Gudjon JP, Anderson SG, May WJ: A clinical evaluation of the "roll-over test" for pregnancy-induced hypertension, Am J Obstet Gynecol 127:1-3, 1977.) Abstracter's comment: The roll-over test (or supine pressor test) is performed at 28-32 weeks' gestation. The brachial blood pressure is taken in the lateral position, then the patient is turned supine. The pressure is again measured, and the test is positive when there is an increase of at least 20 mm Hg diastolic.