An Anticholinergic Effect of Hexylcaine on Airway Smooth Muscle

Hall Downes, M.D., Ph.D.,* and Robert W. Loehning, M.D., Ph.D.†

Hexylcaine, an ester-type local anesthetic, was studied in guinea pig tracheal chains for its comparative effects on intrinsic tone and on responses to carbamylcholine, histamine and isoproterenol. Drug effects were recorded isotonically at a bath pH of 7.5 (CO_2, 5 per cent, and 25 mM bicarbonate) or pH 6.75 (CO_2, 5 per cent, and 5.75 mM bicarbonate), corresponding, respectively, to nonionized drug concentrations of 5.68 and 1.05 per cent of total hexylcaine. Low concentrations (10^{-5} to 3×10^{-4} M) of hexylcaine produced an apparently competitive antagonism ($pA_2 = 4.95$) of carbamylcholine. The extents of antagonism were not significantly different at the two values of experimental pH, indicating that nonionized drug was not essential to the anticholinergic effect. In contrast, the concentration necessary for relaxation of intrinsic tone changed with pH (10^{-3} M at pH 7.5; 3×10^{-3} M at pH 6.75), indicating that nonionized drug was essential, for access or for action, to the nonspecific relaxant effects of high drug concentrations. Hexylcaine, 3×10^{-4} M (pH 7.5), increased the concentrations of carbamylcholine and histamine needed to produce a half-maximal response by 20.9 times and 3.6 times, respectively, and had no effect on responses to isoproterenol. The authors conclude that hexylcaine has selective and apparently competitive anticholinergic effects that are manifest at clinically relevant concentrations and are mechanistically distinct from the general depressant effects of higher concentrations. (Key words: Airway: trachea. Anesthetics, local: hexylcaine. Histamine. Lung: trachea. Parasympathetic nervous system: anticholinergic. Sympathetic nervous system: sympathomimetic agents; isoproterenol.)

IN THE COURSE of comparing effects of different local anesthetics on an *in-vitro* preparation of airway smooth muscle, the guinea pig tracheal chain, we found that low doses of procaine and tetracaine produced selective relaxation of cholinergically mediated tone that was markedly greater than the effects of similar concentrations of lidocaine and bupivacaine.[1] Prominent anticholinergic effects of low concentrations of procaine[2] or tetracaine[3] had been previously found in tracheal chains obtained from other species. The present study, again using guinea pig tracheal chains, employs another ester-type local anesthetic, hexylcaine, to demonstrate selective and competitive anticholinergic effects at clinically relevant concentrations. Atropine-like effects of local anesthetics on airway smooth muscle are important because of the wide use of local anesthetics for diagnostic procedures in patients who have reactive airway disease and, also, because of their potential therapeutic use as bronchodilator aerosols.[4–7] We chose hexylcaine for these *in-vitro* studies and for ongoing studies of bronchodilator effects in man and intact animals because we wished to test an ester-type drug that was less toxic than tetracaine and more effective as a topical anesthetic than procaine. Hexylcaine (Cyclaine®), which has been in use for topical anesthesia of the airway since 1952,[8–12] fulfills these requirements.[13]

Methods and Materials

Matched tracheal chains were prepared as previously described.[1] In brief, pairs of male guinea pigs, 400–800 g, were sacrificed and the tracheas removed from larynx to carina. Rings were cut alternately from the two tracheas and pooled to form matched sets of three (triplicate) or four (quadruplicate) chains, each seven rings in length and comprised of nearly identical tissues. In a few experiments a pair of chains was prepared from alternate segments of a single trachea. The chains were mounted in 50-ml organ baths at 37.5 C in a Krebs-type solution.[1] In most experiments the bath solution was aerated with CO_2, 5 per cent, and O_2, 95 per cent, which gave a pH of 7.5. To study drug effects at pH 6.75 the bicarbonate concentration was lowered from 25 to 5.75 mmol/l. Bath pH was monitored in a separate chamber using a probe electrode. Contractions were recorded isotonically (Harvard 356 transducer) at a gain of about 14× and against a counterweight of 300 mg. The relatively light counterweight was chosen to allow a greater level of muscle contraction (intrinsic tone) before drug addition and a faster response time after drug addition. The chains were allowed to equilibrate for at least an hour until a stable resting length had been achieved before addition of any drug. Cumulative dose–effect relationships were determined with sufficient time allowed to obtain the maximum effect of each drug concentration. Hexylcaine was tested for its effects on intrinsic tone and for antagonism of carbamylcholine, histamine, or isoproterenol dose–response curves; hexylcaine was added to the bath at least 30 min before testing agonist responses. Experiments lasted three to five hours after the initial period of equilibration; during this time there were no rhythmic contractions or evidence of spontaneous activity other than maintenance of a steady level of intrinsic tone.
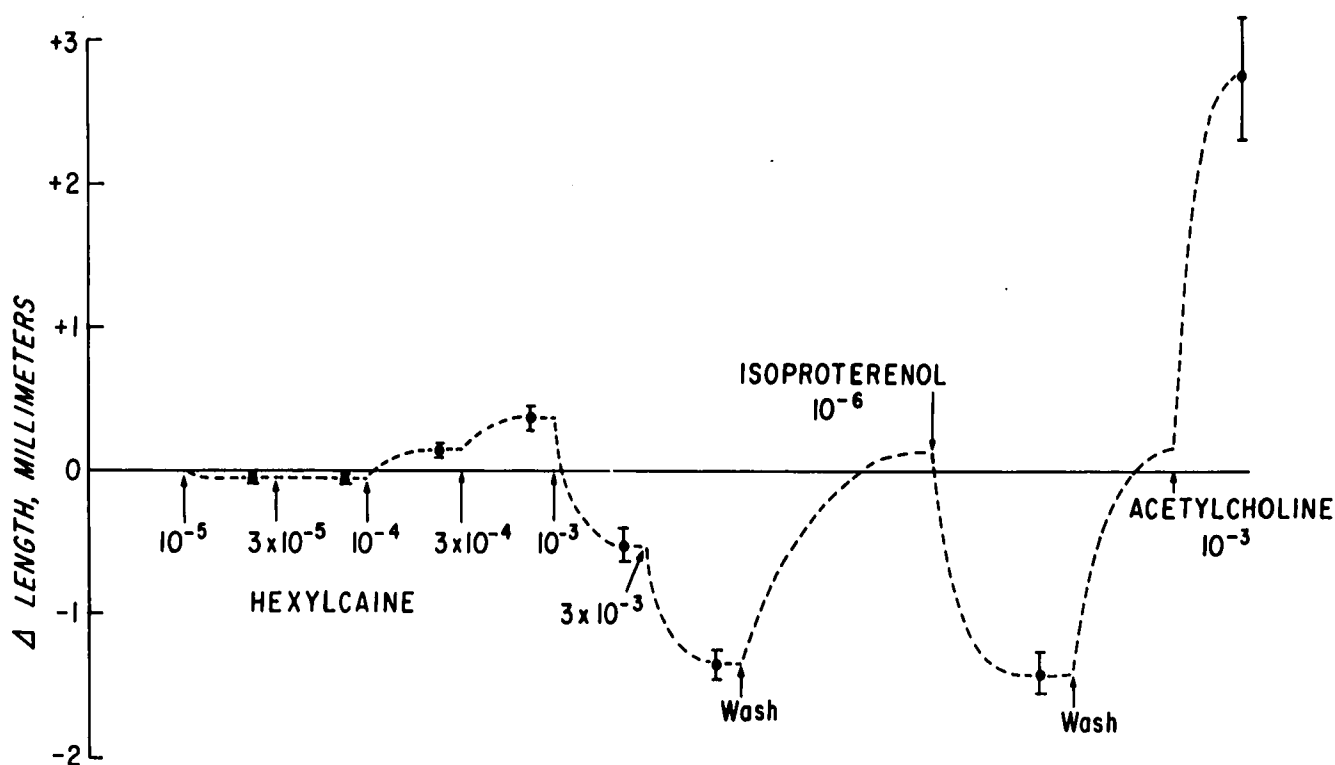
Fig. 1. Effects of hexylcaine on intrinsic tone of tracheal chains at pH 7.5, a semidiagrammatic plot to show the sequence of events and the maximal responses for each drug concentration. Addition of drug is indicated by an arrow with the cumulative drug concentration (M) shown below it. Time span is not indicated since time to maximal effects differed in each experiment. Length change from the pre-hexylcaine baseline is shown on the vertical axis. Positive values indicate mm of contraction, negative values mm of relaxation. Points show the means of five experiments and brackets give the SE.

As in previous experiments,[1] drug effects on intrinsic tone were quantitated in terms of the maximal contraction subsequently elicited by acetylcholine, 10^{-3} M, or the maximal relaxation elicited by isoproterenol, 10^{-6} M. When hexylcaine was used as an antagonist of carbamylcholine, histamine, or isoproterenol, agonist effects were quantitated in terms of their own maximal effects. Carbamylcholine rather than acetylcholine was used as a cholinergic agonist in these experiments to minimize drug hydrolysis in the bath solution. Maximal responses to carbamylcholine were about 5 per cent greater than responses to acetylcholine, 10^{-3} M. The maximal response to carbamylcholine was elicited only once, at the end of the experiment, since this procedure considerably decreased the amplitude of subsequent agonist-induced contractions. Possible hexylcaine effect on the amplitude of the maximal carbamylcholine-induced contraction was tested by comparing the absolute amplitude of contraction in the control chain with contractions in the hexylcaine-treated chains from the same matched set. In addition, hexylcaine, 3×10^{-4} M, was added to the control chain after it had been maximally contracted with carbamylcholine, 10^{-2} M. The

maximal carbamylcholine-induced contraction in the control chains occurred at 10^{-5} M, but carbamylcholine concentration was increased to 10^{-2} M in all chains of the set.

Quadruplicate chains were used to test responses to carbamylcholine at pH 7.5, triplicate chains to test carbamylcholine responses at pH 6.75 and isoproterenol responses at pH 7.5, and paired chains to test histamine responses at pH 7.5. The concentration of agonist needed to produce a half-maximal response (agonist$_{0.5 \text{ max}}$) was calculated from the regression line of the log dose–response curve using all points between 10 and 90 per cent of the maximal response. Slope was calculated from the same regression line. Dose ratios[14] for each set of matched chains were calculated as:

$$\frac{\text{Agonist}_{0.5 \text{ max}} \text{ after hexylcaine}}{\text{Agonist}_{0.5 \text{ max}} \text{ in control chain}}$$

A dose ratio of 2 means that after pretreatment with hexylcaine, twice as much agonist was needed to produce the same effect. For a competitive antagonist,[15] the concentration of antagonist that produces a

dose ratio of 2 is equivalent to the apparent dissociation constant of the antagonist. The negative log of this antagonist concentration is the pA_2[16]; the pA_2 for hexylcaine as an antagonist of carbamylcholine was determined from the regression line for log (dose ratio − 1) as a function of the negative log of antagonist concentration.[17]

Slopes of agonist dose−response curves, maximal responses, and dose ratios after hexylcaine pretreatment were compared with values for control chains using the two-tailed t test for paired data. Differences between these values for carbamylcholine at pH 7.5 relative to histamine at pH 7.5 or carbamylcholine at pH 6.75 were compared using the two-tailed t test for nonpaired data since the chains were obtained from different animals. $P < 0.05$ was considered significant.

All drugs were dissolved in 0.9 per cent sodium chloride solution and were added to the organ baths in increments of 0.5 ml or less. Crystalline hexylcaine hydrochloride was a gift of Merck, Sharp, and Dohme. To calculate the concentrations of ionized and nonionized drug, the experimentally determined pK_a of hexylcaine of 9.08 at 20 C[18] was corrected by the formula of Albert and Serjeant[19] for the effect of increased temperature. This correction gave a pK_a of 8.72 for 37.5 C.

Results

At low concentrations (10^{-5} and 3×10^{-5} M) hexylcaine either had no effect on intrinsic tone or produced barely detectable relaxation (fig. 1; table 1). Progressively increasing the concentration (10^{-4} M) elicited a slight contracture followed by marked relaxation occurring abruptly as a "breakpoint" (10^{-3} M). In contrast to findings in previous experiments with lidocaine,[1] the extent of hexylcaine-induced contracture was less in the more acidic bath solution (table 1); however, the preceding low-dose relaxation was also more pronounced at the more acidic pH and may have partially obscured the contracture.

The major pH-dependent difference in hexylcaine effects was in the breakpoint concentrations, which, as with lidocaine, were consistently higher at the more acidic pH. Thus, hexylcaine, 10^{-3} M, produced a slight contracture at pH 6.75 but marked relaxation at pH 7.5 (table 1). Maximal responses to acetylcholine or isoproterenol were not significantly different at the two values of pH (table 1).

Since the tracheal chains possessed substantial intrinsic tone, low doses of hexylcaine could be tested against either the contractor or relaxant effects of other drugs. Low concentrations of hexylcaine had no effect on isoproterenol-induced relaxation (fig. 2) but caused a dose-related shift to the right of carbamylcholine dose−response curves (fig. 3). Even at the lowest concentration (10^{-5} M), hexylcaine significantly increased the dose of carbamylcholine needed to elicit a half-maximal response, and carbamylcholine dose ratios were significantly different at different concentrations of hexylcaine (table 2). Mean carbamylcholine dose ratios were higher at the more acidic pH, but the difference was not statistically significant (table 2).

Although low doses of hexylcaine markedly shifted the carbamylcholine dose−response curve, they had little effect on the slope of the log dose−response curve or on the maximal response (table 2). The slope was significantly increased by hexylcaine at 3×10^{-4} M (pH 7.5 and pH 6.75) and at 5.5×10^{-5} M (pH 7.5), but the effect was very slight (table 2; fig. 3). Maximal responses to carbamylcholine in chains pretreated with hexylcaine were not significantly different from maximal responses in control chains, but addition of hexylcaine, 3×10^{-4} M, to the control chain, after it had been maximally contracted with carbamylcholine, always produced slight relaxation. The maximal carbamylcholine-induced responses in control chains after addition of hexylcaine, 3×10^{-4} M, were 92 ± 3 per cent of the pre-hexylcaine value in experiments at pH 7.5 and 92 ± 1 per cent of the pre-hexylcaine value in experiments at pH 6.75 (mean $\pm$ SE). In-

TABLE 1. Effects* of Increasing Hexylcaine Concentrations on Intrinsic Tone at pH 7.50 and at pH 6.75

	Low Dose, Relaxation		Intermediate Dose, Contracture			High Dose, Relaxation		Maximal Effects (mm)†	
	10^{-5} M	3×10^{-5} M	10^{-4} M	3×10^{-4} M	10^{-3} M	10^{-3} M	3×10^{-3} M	Acetylcholine 10^{-3} M	Isoproterenol 10^{-6} M
pH 6.75	-4.5 ± 1.2	$-7.7 \pm 2.8‡$	$1.5 \pm 0.4‡$	$5.5 \pm 1.2‡$	$10.5 \pm 3.2‡$	—	-89.9 ± 4.1	3.51 ± 0.62	-1.61 ± 0.35
pH 7.50	-2.5 ± 1.1	-2.5 ± 1.1	6.1 ± 1.0	14.8 ± 2.2	—	$-51.4 \pm 10.6†$	-96.5 ± 3.9	2.71 ± 0.42	-1.42 ± 0.13

* Percentage of maximum. Calculated as change in length from immediately preceding state; contracture is expressed as (+) percentage of the maximal contraction as subsequently elicited by acetylcholine; relaxation is expressed as (−) percentage of the maximal relaxation as subsequently elicited by isoproterenol. Mean $\pm$ SE, n = 5. Absolute amplitudes of the maximal effects are given

in the columns on the right.

† Change in length (mm) from pre-hexylcaine control value. Positive values indicate mm of contraction, negative values mm of relaxation. Mean $\pm$ SE, n = 5.

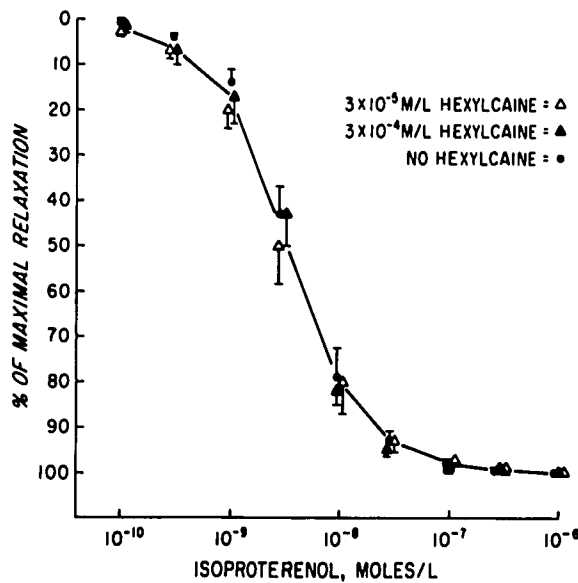‡ Significant difference between values obtained at pH 6.75 and pH 7.5.

FIG. 2. Cumulative dose-response relationships for isoproterenol-induced relaxation of intrinsic tone of matched sets of tracheal chains in the absence of hexylcaine (●), and after addition of hexylcaine 3×10^{-5} M (Δ) or 3×10^{-4} M (▲). Points show the means of five experiments and brackets give the SE. The three isoproterenol dose-response curves are superimposable and are represented by a single line.

TABLE 2. Effects of Hexylcaine on Carbamylcholine Dose-response Characteristics

	Hexylcaine (mol/l)	Ratio* of Slopes	Ratio† of Maximal Responses	Dose Ratio‡
pH 7.50	10^{-5}	1.16 ± 0.27	1.17 ± 0.51	1.80 ± 0.78
	5.5×10^{-5}	1.16 ± 0.10	1.12 ± 0.56	5.06 ± 3.08
	3×10^{-4}	1.21 ± 0.23	1.00 ± 0.37	20.94 ± 11.67
pH 6.74	5.5×10^{-5}	1.21 ± 0.18	1.07 ± 0.51	7.46 ± 4.13
	3×10^{-4}	1.27 ± 0.12	0.90 ± 0.41	29.28 ± 15.31

* Slope of carbamylcholine log dose-response curve;

$$\frac{\text{slope in hexylcaine-treated chain}}{\text{slope in control chain}},$$

mean of ratios ± 95 per cent confidence limits (CL).

† Mm of contraction elicited by carbamylcholine (10^{-2} M);

$$\frac{\text{response in hexylcaine-treated chain}}{\text{response in control chain}},$$

mean of ratios ± 95 per cent CL.

‡ $\dfrac{\text{Agonist}_{0.5\,max}\text{ in hexylcaine-treated chain}}{\text{Agonist}_{0.5\,max}\text{ in control chain}},$

mean of ratios ± 95 per cent CL.

creasing the concentration of hexylcaine tenfold (3×10^{-3} M), however, decreased the carbamylcholine maximal response to less than 10 per cent of its pre-hexylcaine value.

Hexylcaine, 3×10^{-4} M, also produced a shift to the

right of the histamine dose-response curve (fig. 4). The histamine dose ratio was 3.63 ± 0.13 (±95 per cent confidence limits [CL]), which is significantly less than the carbamylcholine dose ratio for an equivalent hexylcaine concentration (table 2). Ratios (±95 per cent CL) of slopes and maximal responses for histamine dose-response curves in hexylcaine-treated chains compared with control chains were 1.21 ± 0.09 and 1.00 ± 0.31, respectively. As in experiments with carbamylcholine, the slight increase in slope was statistically significant. Maximal responses to histamine occurred at 10^{-4} M in control chains and at 3×10^{-4} M in hexylcaine-treated chains; higher concentrations of histamine produced relaxation rather than further contraction. Maximal responses to histamine ranged from 58 to 75 per cent of the maximal response to carbamylcholine as subsequently elicited after washout of other drugs.

Discussion

Vagally mediated reflexes play a major role in the initiation of bronchospasm,[20-22] and much of the observed bronchodilator effect of local anesthetic aerosols[4-7] probably reflects interruption of reflex bronchospasm. With amide-type local anesthetics, reflex block occurs through topical anesthesia of airway sensory receptors and vagal efferent fibers.[5] Our results suggest that ester-type local anesthetics have an additional atropine-like action that could significantly contribute to bronchodilator effect.

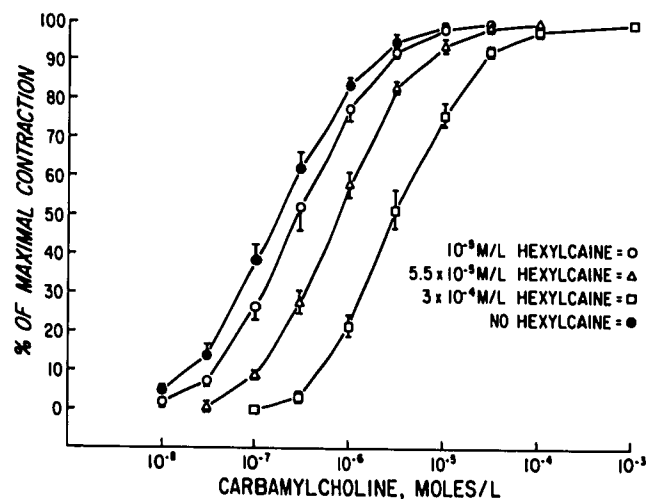Fleish and Titus,[3] using rat tracheal chains, demon-

FIG. 3. Cumulative dose-response relationships for carbamylcholine-induced contraction of matched sets of tracheal chains in the absence of hexylcaine (●) and after addition of hexylcaine 10^{-5} M (O), 5.5×10^{-5} M (Δ) or 3×10^{-4} M (□). Points show the means of five experiments and brackets give the SE.

strated parallel displacement of carbamylcholine dose–response curves by low doses of tetracaine. On this basis and because the additive anticholinergic effects of atropine and tetracaine fulfilled the theoretical expectations for drugs acting on the same receptor, they felt that the anticholinergic effect of tetracaine might represent a true competitive antagonism at the cholinergic receptor or at an allosteric site on or near the receptor macromolecule. Our data provide further support for such an action of ester-type local anesthetics on the muscarinic receptors of airway smooth muscle. An ideal competitive antagonist as described by mass-action laws produces a shift to the right of the agonist dose–response curve without change in its slope or maximal response, and with a regression between log (dose ratio − 1) and minus log antagonist concentrations that is linear with a slope of minus one.[15,17] Low doses of hexylcaine (10^{-5} to 3×10^{-4} M) tested against carbamylcholine came close to fulfilling these criteria (table 2; figs. 3 and 5), although there were minor changes in slope and maximal response. The regression for log (carbamylcholine dose ratio − 1) as a function of minus log hexylcaine concentration (fig. 5) was linear, with a slope not significantly different from minus one. The pA_2 as determined from the horizontal intercept of figure 5 was 4.95, corresponding to an apparent dissociation constant of 1.1 × 10^{-5} M. The apparent affinity of hexylcaine for the muscarinic receptor is thus about one ten-thousandth that of atropine.[17] Nevertheless, clinical doses of hexylcaine, 3 to 5 ml of 5 per cent solution ad-

Fig. 5. Carbamylcholine dose ratio (DR) as a function of hexylcaine concentration (see Methods) at pH 7.5 (●) and pH 6.75 (O). The horizontal intercept of the regression line for log (DR-1) vs (−)log hexylcaine concentration gives a pA_2 of 4.95 ± 0.20 (±95% CL) for experiments at pH 7.5 and a pA_2 of 5.12 ± 0.39 for experiments at 7.5; the corresponding slopes ± 95% CL are −0.86 ± 0.21 and −0.89 ± 0.38.

ministered as an intratracheal spray, produced peak blood concentrations of 10^{-5} to 3×10^{-5} M.‡ Similar concentrations in vitro decreased cholinergic responses (fig. 3) with virtually no effect on intrinsic tone (fig. 1).

The selectivity of hexylcaine as a cholinergic antagonist can be assessed by comparing effects of hexylcaine 3×10^{-4} M on responses to carbamylcholine, isoproterenol and histamine. The isoproterenol dose–response curve was unaltered (fig. 2), the histamine dose–response curve was shifted to the right by a dose ratio of 3.63, and the carbamylcholine dose–response curve was shifted to the right by a dose ratio of 20.94. By extrapolation from figure 5, hexylcaine, 3.4×10^{-5} M, would produce a carbamylcholine dose ratio of 3.63, which is equivalent to the observed histamine dose ratio at hexylcaine 3×10^{-4} M. Hexylcaine is thus about nine times more potent as an antagonist of carbamylcholine than as an antagonist of histamine.

The dissociation between the selective anticholinergic effects of low doses of hexylcaine and the
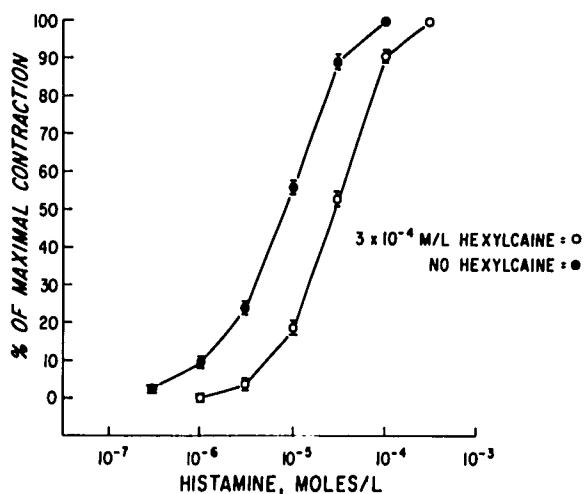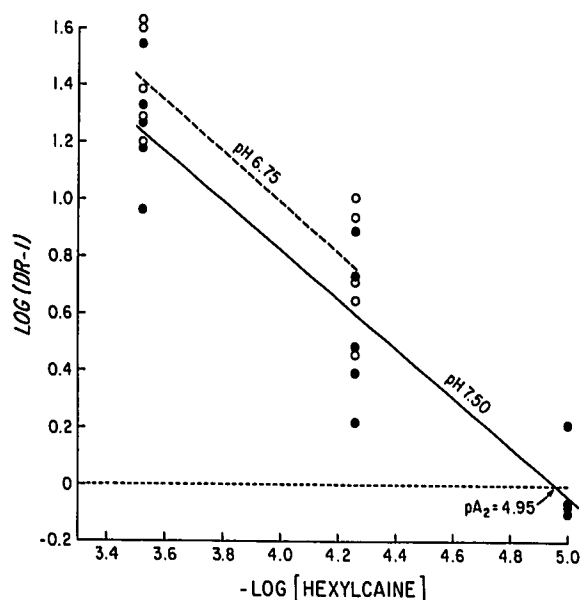

Fig. 4. Cumulative dose-response relationships for histamine-induced contraction of matched sets of tracheal chains in the absence of hexylcaine (●) and after addition of hexylcaine 3×10^{-4} M (O). Points show the means of four experiments and brackets give the SE.

nonspecific relaxant effects of high doses is emphasized by results of experiments conducted at different values of bath pH. Decreasing pH from 7.5 to 6.75 decreased the concentration of nonionized drug from 5.68 to 1.05 per cent of total drug. Since lowering the pH caused a five-fold decrease in concentration of nonionized drug, effects dependent on nonionized drug should have been proportionately decreased. The nonspecific relaxant effect, as manifested by the breakpoint concentration, was decreased at the more acidic pH (table 1), whereas the anticholinergic effect was not significantly altered. Since the test procedure used to determine the anticholinergic effect was clearly capable of distinguishing a fivefold change in drug concentration, we conclude that nonionized drug is not essential to the anticholinergic effect. This is compatible with an action on muscarinic receptors, which are superficial and readily blocked by a variety of quaternary derivatives of atropine.[23]

The extent of low-dose anticholinergic effects of ester-type local anesthetics appears to be unrelated to their potencies and toxicities as local anesthetics. Thus, in previous experiments,[1] procaine, 3×10^{-5} M, and tetracaine, 3×10^{-5} M, were equally effective in relaxing carbamylcholine-induced contraction of tracheal chains. In contrast, breakpoint concentrations (procaine[1] $\geq 6 \times 10^{-3}$ M; hexylcaine 10^{-3} M; tetracaine[1] $\leq 3 \times 10^{-4}$ M) are inversely related to the local anesthetic potencies and systemic toxicities determined in nerve and in intact animals.[13] The simplest explanation is to assume that ester-type local anesthetics act at two distinct sites on airway smooth muscle. One site is superficial and closely associated with or identical to the muscarinic receptor. Differences in chemical structure that markedly influence local anesthetic potency do not necessarily affect action at this site. The other site requires nonionized drug, either for passage across a membrane or as the active moiety. Activity at this site seems to parallel activity as a local anesthetic and is probably responsible for the nonselective[24] and noncompetitive[25] relaxant effects observed in other studies.

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