The Effect of a Local Anesthetic, Lidocaine, on Guinea Pig Trachealis Muscle In Vitro[1-3]

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SUMMARY

The effect of lidocaine was studied in guinea pig trachealis muscle by dose-response reversal and protection of agonist-induced contractures in a superfusion system. Lidocaine reversed histamine, acetylcholine, and depolarizing hypertonic potassium contractures with the median effective doses of 2.8, 6.0, and 3.2 mg. Lidocaine presuperfusion shifted in a nonparallel fashion ($P < 0.05$) the dose-response of histamine, acetylcholine, depolarizing potassium, and supramaximal electrical stimulation by contact electrodes. Pretreatment with 7×10^{-6} *M* atropine did not modify lidocaine's inhibition of the hypertonic potassium contractions. These findings and the decrease in maximal response indicated noncompetitive antagonism. In contrast to isoproterenol, the action of lidocaine was not influenced by β-blockade induced by superfusate propranolol, 1.0 μg per ml ($P = 0.2$). Lidocaine's effect on trachealis smooth muscle was facilitated by a decrease in hydrogen ion activity from pH 6.71 to 7.90, consistent with enhanced penetration of the free base. Low bolus dose lidocaine-induced contractures were noted in many studies. The potency of isoproterenol in comparison to lidocaine, as indexed by median effective doses, was 10^5 greater for reversal of histamine contractures and 10^4 greater for acetylcholine. The data were consistent with a nonspecific, reversible antagonism on the smooth muscle cell and may involve an effect on calcium activity.

Introduction

The chemical compounds clinically employed as local anesthetics are secondary or tertiary amines capable of blocking nerve excitation and conduction in a reversible manner. Their electrophysiologic action in peripheral nerves is by reduction of the transient sodium ion conductance changes necessary for action potential generation, an action that appears linked to membrane displacement of calcium (1–3). Suppression of ventricular arrhythmias is based on a similar reduction in the rate of increase of the cardiac muscle action potential and thus the depolarization process. The reader is referred to many comprehensive publications concerning these clinical applications and their physiologic basis (1, 2, 4, 5).

Recent studies indicate that local anesthetics can antagonize contraction in a variety of muscle tissues, smooth and striated: rabbit and rat aorta (6, 7); guinea pig and cat atria (7); frog and rabbit skeletal muscle (8, 9); rabbit and guinea pig taenia coli, rat uterus (10). The mechanism here is generally ascribed to (*1*) a direct stabilizing effect on the muscle membrane by the reversible interference of transmembrane ionic permeability, i.e., conductance of sodium and potassium ions, and (*2*) at the intracellular level an uncoupling of the excitation-contraction process by means of an antagonism of the

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calcium fluxes that link the two processes (8, 10–13).

Studies on the effects of local anesthetics on airway smooth muscle have been limited (6, 14). This study evaluated specifically the effects of one local anesthetic, lidocaine, on the isolated trachealis smooth muscle of the guinea pig utilizing a dose-response analysis of lidocaine-agonist interactions.

Materials and Methods

Male guinea pigs (A & E Farms, Altamont, N. Y.) weighing 300 to 600 g were killed by stunning and bleeding. Each trachea was quickly removed, dissected free of extraneous tissue, and cut spirally (15). This resulted in an alternating pattern of cartilage and smooth muscle, and each experimental preparation consisted of such a cartilage-muscle-cartilage strip of trachea. One cartilagenous end was fastened with #50 cotton thread to a fixed clamp, and the other via a thin piece of stainless steel wire to a Grass FT03C Force Displacement Transducer. Isometric changes in tension were recorded on a calibrated Hewlett-Packard 7786A Thermal Tip Recording System.

Preparations were equilibrated in our modified superfusion system to a steady baseline of 2.0 g (± 0.5 g) initial tension, requiring a mean time of 60 min. Stable active tension was defined by repeated agonist application until a constant response within ± 5 per cent was achieved. All muscle strips were observed for the rate of active tension development, and those that showed instability of baseline and/or inconsistent development of tension to a given dose were eliminated from the study. This procedure eliminated abnormally functioning muscle strips (16, 17). The Krebs Henseleit superfusate consisted of the following: NaCl, 118.1 m*M*; KCl, 4.7 m*M*; $NaHCO_3$, 24.8 m*M*; $MgSO_4 \cdot 7\ H_2O$, 2.4 m*M*; KH_2PO_4, 1.2 m*M*; $CaCl_2$, 2.5 m*M*; glucose, 10.0 m*M* in distilled, deionized water. Distilled, deionized water was used throughout the experiments for the preparation of superfusion media and agonist solutions. Superfusion was maintained at 37° C by passage through a heated condenser and equilibrated with 95 per cent O_2 and 5 per cent CO_2. Po_2 and Pco_2 and pH of the superfusate were monitored daily with a 313 Blood Gas Analyzer (Instrumentation Laboratories, Inc.). Partial pressures for CO_2 ranged from 35 to 45 mm Hg and approximately 400 mm Hg for O_2 with an average pH of 7.45. Superfusate flow of 5.0 ml per min was maintained by Buchler polystatic pump from a 2-liter reservoir of Krebs Henseleit.

Antagonists

The action of lidocaine HCl monohydrate (Astra Pharmaceutical Products, Inc.) as an antagonist was studied in two ways, protection of agonist-induced contraction and reversal from agonist-induced contracture. In the protection studies, lidocaine was dissolved in the superfusate in concentrations (free base) ranging between 0.25 and 2.0 mg per ml. Each lidocaine-containing Krebs Henseleit solution was allowed to superfuse the muscle for 30 min before the onset of the next experiment. Responses to agonists were obtained before and after the lidocaine–Krebs Henseleit superfusion with the same muscle serving as its own control. Upon completion of an experiment, a 60-min washout period (Krebs Henseleit alone) was followed by reapplication of the agonist to test the viability of the muscle, defined as a contraction of at least 90 per cent of the original control response.

Reversal studies tested the action of lidocaine against a sustained agonist-induced contracture. This was produced by a 30-min agonist–Krebs Henseleit superfusion to a steady state. Then, bolus doses of lidocaine from 0.5 to 16.0 mg per 0.2 ml were delivered during a 5-sec interval into the superfusate at the top of the heating condenser. Responses were measured as maximal change in tension (grams) to milligrams or micrograms applied. As a control, 200 µl of distilled water were similarly delivered; this did not alter the observed tension in any case. Isoproterenol reversal was also conducted on each muscle strip under identical conditions for paired comparisons to lidocaine. DL-isoproterenol hydrochloride (Sigma Chemical Company) was dissolved in double distilled, deionized water to final concentrations (expressed as free base) between 0.005 and 0.32 µg per 200 µl. The doses of lidocaine and isoproterenol were random.

Agonists

Pharmacologic. The ability of lidocaine to protect against chemical agonist-induced contractions was studied using histamine dihydrochloride and acetylcholine chloride. Histamine (USP) and acetylcholine were prepared in concentrations between 1.5 and 1,024 µg per 200 µl (expressed as the weight of the free base), and 200-µl bolus doses injected as described in the previous section. During reversal studies, histamine and acetylcholine were dissolved in the Krebs Henseleit reservoir to a final concentration of 1.0 µg per ml each.

Ionic potassium. During protection studies, increasing potassium concentrations of 10, 20, 40, and 80 m*M* were selected as ionic depolarizing agonists, the osmolarity maintained by replacing potassium chloride for sodium chloride on an equimolar basis. Lidocaine was added to both the superfusion reservoir and the high potassium infusions to maintain the proper concentration throughout, these introduced at 5 ml per min to the condenser for 30 sec by an infusion pump (Sage). For reversal studies, which included isoproterenol and lidocaine, the potassium concentration of the superfusion reservoir was 80 m*M*.

The protective action of lidocaine, 0.25 mg per ml superfusate concentration, was also tested in the

presence of a continuous atropine blockade (5.0 μg per ml [7×10^{-6} *M*] superfusate concentration) to application of depolarizing potassium solutions of 10, 20, and 40 m*M*; these were applied for 60 sec. Equipotent acetylcholine contractures to 512 μg before and at the termination of the study verified the viability of the muscle, with atropine causing a mean of 75 per cent antagonism to this dose.

Electrical stimulation. Muscle preparations were stimulated transmurally with supramaximal square wave dc pulses (amplitude 80 volts, duration 3 msec) generated by a Grass S8B stimulator and delivered via 2 platinum wire electrodes directly to the muscle. Pulses were applied for 10 sec at frequencies of 10, 20, and 40 Hz. Responses were recorded as number of grams of contraction before and after the addition of lidocaine (1.0 mg per ml) to the superfusate.

β-adrenergic receptor blockade. Propranolol was employed to determine the effect of β receptor blockade on both lidocaine-induced reversal and isoproterenol-induced reversal of a sustained histamine-induced contracture. Responses to lidocaine and to isoproterenol were obtained before and during β receptor blockade. Data were analyzed with "Student's" t test, paired comparisons.

Pharmacologic agonists and antagonists were added to the superfusion reservoir in the following concentrations (expressed as free base): histamine, 1.0 μg per ml; lidocaine, 1.0 mg per ml; isoproterenol, 1.0 μg per ml; propranolol, 1.0 μg per ml. Lidocaine and isoproterenol concentrations were determined previously to be equipotent. A sustained contraction induced by a Krebs Henseleit histamine superfusion was maintained for 30 min before onset of the experiment.

Cholinergic receptor blockade. For the standardization of cholinergic dose-response analysis, atropine was employed in a final concentration of 0.5 μg per ml as a standard competitive antagonist to acetylcholine contractions.

pH studies. Superfusate hydrogen ion concentrations were varied by addition of 1.0 *N* NaOH and 1.0 *N* HCl resulting in pH of 6.71, 7.32, and 7.90 in Krebs Henseleit. Response to histamine (8.0-μg bolus dose) was examined before and after lidocaine.

Statistical Analysis

Dose-response data were analyzed for competitiveness by regression analysis for determination of slope and linearity. Slopes were compared by a "Student's" t test utilizing a pooled residual mean square with a P value ≤ 0.05 for significance. Two population regression coefficients were also analyzed by variance ratio tests (18). "Student's" t test was also used for difference of means. All calculations were computed by a Hewlett-Packard Model 9810A Calculator and Hewlett-Packard statistical programs. Dose-response data were plotted as semi-log and double-reciprocal (Lineweaver-Burk) graphs for determination of competitiveness. Estimates of median effective dose (ED_{50}) in the lidocaine and isoproterenol reversal studies were determined visually. Reversal studies are plotted as per cent relaxation of the agonist-induced contraction versus unit dose applied.

Results

Reversal by Lidocaine and Isoproterenol of Histamine, Acetylcholine, and Hypertonic Potassium-Induced Contractions

In figure 1, the effect of bolus doses of lidocaine and of isoproterenol on a sustained contraction of acetylcholine, histamine, and hypertonic potassium is presented; concentrations of acetylcholine and histamine were equipotent ($0.5 > P > 0.4$). Data are expressed as per cent of maximal relaxation with values representing mean ± 1 SE in 5 animals. A low-dose bolus of 0.5 mg of lidocaine caused an average 5 per cent increase in tension to histamine and a 3 per cent mean increase with hypertonic potassium. Higher doses of lidocaine (> 0.5 mg) exhibited a dose-dependent relaxation with complete reversal of all agonist-induced contractions. Application of isoproterenol also yielded similar dose-dependent reversals of each agonist. The bolus doses required for complete relaxation against histamine, acetylcholine, and hypertonic potassium were 8.0 mg, 16.0 mg, and 16.0 mg for lidocaine, and 0.04 μg, 0.32 μg, and 3.2 μg for isoproterenol, respectively.

The ED_{50} of lidocaine and of isoproterenol for reversal of a histamine-induced contraction was 2.8 mg and 0.01 μg, respectively, and for

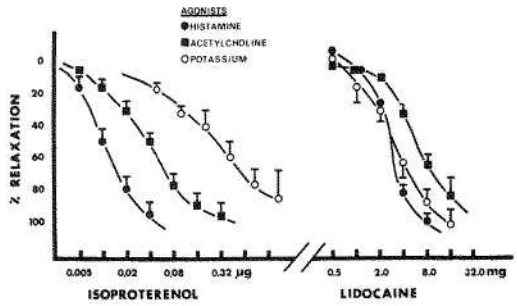

Fig. 1. Reversal studies. Semi-log dose-response curves for isoproterenol and lidocaine reversal of sustained contractions by histamine (1.0 μg per ml), acetylcholine (1.0 μg per ml), and hypertonic potassium (80 m*M*). Each point = mean $\pm$ 1 SE, n = 5 animals. ED_{50} were for lidocaine: histamine, 2.8 mg; acetylcholine, 6.0 mg; potassium, 3.2 mg; for isoproterenol: histamine, 0.01 μg; acetylcholine, 0.04 μg; potassium, 0.26 μg.

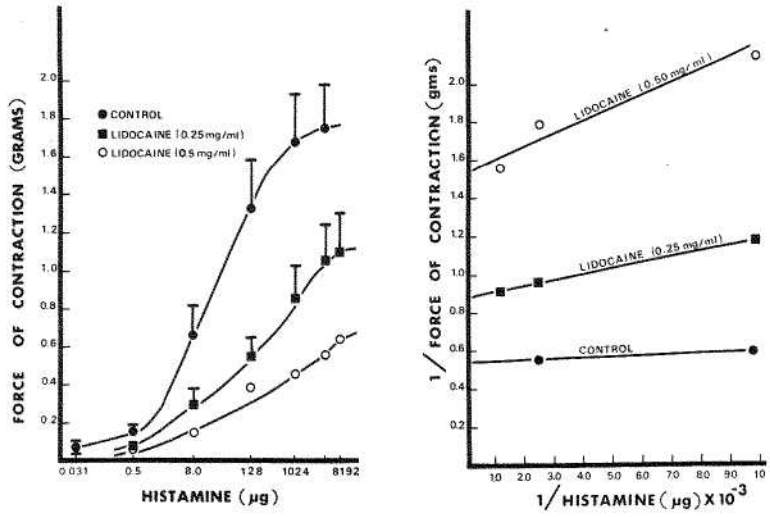

Fig. 2. A. Lidocaine protection to histamine. Semi-log dose-response curves for histamine against lidocaine of 0.25 mg per ml and 0.50 mg per ml. Mean ± 1 SE, n = 5 animals. A decrease in maximal response and nonparallel shift ($P < 0.05$) indicates noncompetitive antagonism. Mean data points for lidocaine (0.50 mg per ml) includes the standard error. B. Protection of maximal histamine response. Double reciprocal (Lineweaver-Burk) analysis of histamine contractures with lidocaine of 0.25 mg per ml and 0.50 mg per ml. Mean in 5 animals. The intercept indicates maximal response before and with lidocaine; see text.

acetylcholine was 6.0 mg and 0.04 μg, respectively. Both lidocaine and isoproterenol reversed hypertonic potassium-induced contractions, with an ED_{50} of 3.2 mg for lidocaine and 0.26 μg for isoproterenol. Thus, isoproterenol was approximately 10^5 more effective in reversing histamine- and acetylcholine-induced contractions, and 10^4 more effective in reversal of hy-

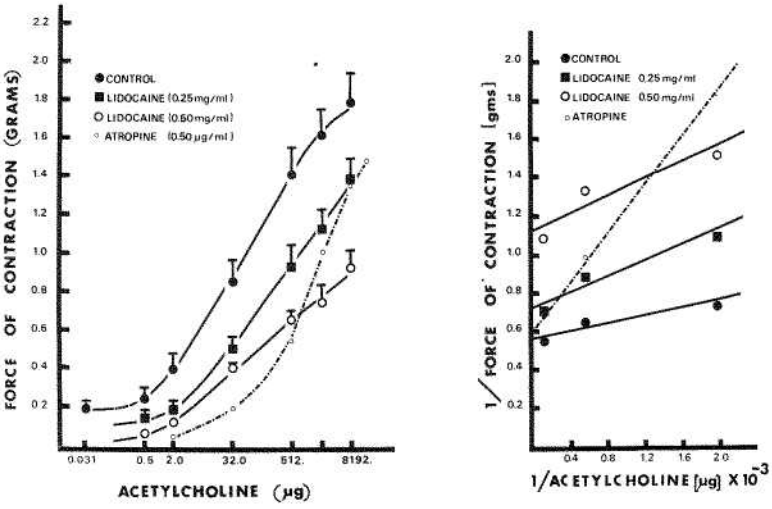

Fig. 3. A. Lidocaine protection of acetylcholine contractions. Semi-log dose-response curves for the contractile action of acetylcholine in the presence of lidocaine of 0.25 mg per ml and 0.50 mg per ml, and atropine of 0.50 μg per ml. Each point is the mean ± 1 SE, n = 5 animals. Atropine exhibits competitive antagonism, P = 0.05. Lidocaine competition is noncompetitive. B. Protection study to acetylcholine: maximal response. Double reciprocal plot for acetylcholine at lidocaine doses of 0.25 mg per ml, 0.50 mg per ml, and atropine of 0.50 μg per ml; n = 5 animals. Atropine maintains the same maximal response and parallel shift of figure 3A, corroborating competitive antagonism; see text.

pertonic potassium-induced contractions than lidocaine under identical isometric tensions in paired comparisons.

The Effect of Lidocaine on Histamine, Acetylcholine, Potassium, and Electrical Stimulation: Protection Studies

Histamine. A standard dose-response curve to histamine was attained by the method outlined. Concentrations of 0.25 and 0.5 mg per ml of lidocaine significantly decreased the slope from 0.46 to 0.26 and 0.15 ($P < 0.05$), respectively, with a reduction in maximal contractile response (figure 2A). This nonparallel rightward shift and reduction in maximal response characterizes a noncompetitive type of inhibition of histamine. A double reciprocal plot (figure 2B) shows a change in Δ-maximum but an apparently unchanged K_X (dissociation constant) (not shown), traditionally interpreted as noncompetitive inhibition.

Acetylcholine. The effect of atropine on contractile responses of the guinea pig trachealis muscle to acetylcholine was similar to other types of excitable tissue, and 0.5 μg per ml of atropine shifted the acetylcholine dose-response curve to the right, maintaining a parallel slope ($P = 0.05$) (figure 3A) (10, 12).

Lidocaine (0.25 mg per ml) caused a nonparallel rightward shift of the acetylcholine dose-response curve, reducing the slope from 0.42 to 0.31 ($0.01 > P > 0.001$). Lidocaine (0.5 mg per ml) further shifted the acetylcholine dose-response curve to the right and reduced the slope to 0.22 ($0.02 > P > 0.01$). The double reciprocal plot also confirms the noncompetitive inhibition by lidocaine versus the competitive inhibition by atropine to cholinergic stimulation (figure 3B).

Electrical stimulation. The effect of lidocaine (1.0 mg per ml) during direct electrical stimulation resulted in essentially complete abolition of contraction (figure 4). All treatments were significantly different from control ($P < 0.01$) according to "Student's" t test, paired comparisons.

Potassium. The results of dose-response experiments conducted with osmotically balanced hypertonic potassium Krebs Henseleit solutions are shown in figure 5A. A dose-dependent reduction by lidocaine (0.25 mg per ml and 0.5 mg per ml) was observed at each potassium concentration ($P < 0.05$). In the presence of 7×10^{-6} *M* atropine, lidocaine (0.25 mg per ml) still produced a significant protection against

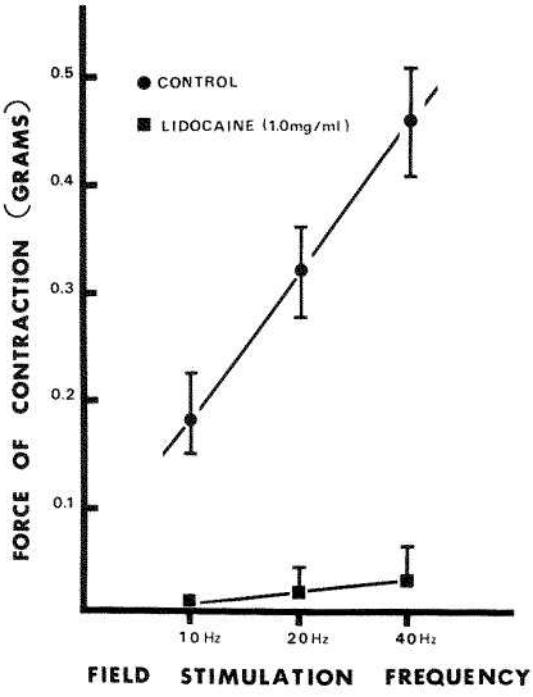

Fig. 4. DC electrical stimulation. Semi-log dose-response curves for the contractile effect of direct muscle supramaximal square wave pulse stimulation in the presence of lidocaine (1.0 mg per ml). Each point represents the mean ± 1 SE, n = 5 animals.

the 3 selected potassium concentrations ($P < 0.05$) (figure 5B). The relative per cent of inhibition at a given K^+ concentration was constant (50 per cent at 40 m*M*).

The Effect of β-adrenergic Blockade and pH

β-blockade. In the studies conducted to evaluate the influence of lidocaine on β receptor activity, lidocaine-induced relaxation was unchanged after the addition of propranolol to the superfusate ($P = 0.2$), whereas isoproterenol was significantly reduced ($0.02 > P > 0.01$) (figure 6).

pH. Responses to histamine were not significantly different ($P > 0.05$) at pH 6.71, pH 7.32, and pH 7.90. The efficacy of lidocaine, however, was significantly greater at pH 7.90 ($P < 0.05$) (figure 7).

Discussion

Local anesthetics have been reported to be effective antagonists to a variety of stimuli in striated muscle and smooth muscle of vascular and uterine origin (6, 10, 13). In this study the action of lidocaine was tested in guinea pig trachealis

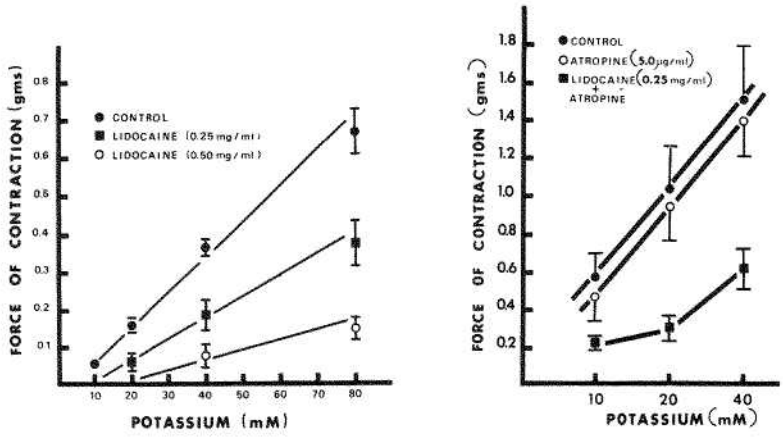

Fig. 5. Hypertonic potassium depolarization. A. Dose-response of a 30-sec exposure to hypertonic potassium versus lidocaine at 0.25 mg per ml and 0.50 mg per ml. Each point is the mean ± 1 SE, n = 5 animals. Lidocaine protection was significant in both doses, $P < 0.05$. B. Dose-response of a 60-sec exposure to hypertonic potassium in the presence of atropine (5.0 μg per ml) and lidocaine (0.25 mg per ml) plus atropine. Atropine did not significantly alter the potassium dose-response curve ($0.6 > P > 0.5$), where lidocaine plus atropine reduced the response significantly ($P < 0.01$).

muscle against a variety of smooth muscle agonists, including histamine, acetylcholine, hypertonic potassium, and electric field stimulation. Evaluation was carried out in two ways. First, we compared lidocaine and isoproterenol as airway smooth muscle relaxants in a precontracted muscle. Both lidocaine and isoproterenol were found to be effective in completely reversing contractions produced by histamine, acetylcholine, and hypertonic potassium. Under our experimental conditions of histamine-induced contractions, the comparative effectiveness of these 2 drugs is reflected in their ED_{50} of 2.8 mg of lidocaine versus 0.01 μg of isoproterenol.

The potency of isoproterenol varied against the 3 cited agonists, as measured by ED_{50}, being most potent against histamine ($ED_{50} = 0.01$ μg) and least potent against hypertonic potassium ($ED_{50} = 0.28$ μg) (figure 1). The potency of lidocaine, however, on each of these agonists was similar and suggests that lidocaine exhibits the same affinity for each agonist receptor site, or that it acts at a step common to all of these excitatory stimuli (10).

Lidocaine, in low concentrations (0.5-mg bolus dose), caused a slight increase in basal tension, above the histamine- and potassium-induced contractions, a biphasic dose-dependent response observed by others (10, 13). Nishimura and associates (19) investigated this phenomenon in relation to vasoconstriction produced by the action of cocaine on vascular smooth muscle, but the precise mechanism of vasoconstriction has not been elucidated (1). In contrast, all doses of isoproterenol used resulted in relaxation.

In the pretreatment (protection) studies, lidocaine was found to act as a noncompetitive antagonist to both histamine- and acetylcholine-induced contractions. Fleisch and Titus (6) and Feinstein and Paimre (12) reported competitive cholinergic antagonism by local anesthetics in low concentrations, i.e., concentrations less than 0.15 mg per ml. Higher concentrations resulted in noncompetitive type shifts in the dose-response curves. This has been viewed as an inhibitory action of local anesthetics, not toward the agonist-receptor interaction, but rather the consequence of their interaction with the receptor site involved in the transmembrane movement of calcium or its release from intracellular stores. Feinstein and Paimre (10) also reported that local anesthetics act as noncompetitive inhibitors toward histamine-induced contractions in rat uterine muscle. This is consistent, therefore, with our findings in guinea pig trachealis muscle tested against histamine and acetylcholine.

In our study, the activity of lidocaine toward histamine-induced contractions was increased by reducing the hydrogen ion concentration. One partial explanation for this pH effect

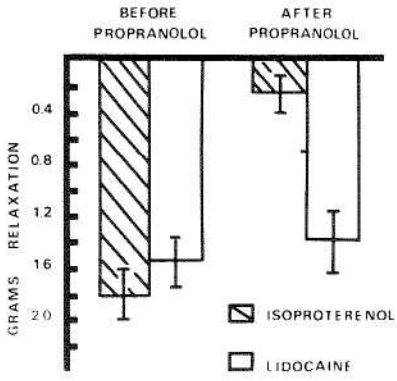

Fig. 6. Effect of propranolol. The effect of propranolol (1.0 μg) on lidocaine (1.0 mg per ml) and isoproterenol (1.0 μg per ml) reversal of histamine-induced contraction; expressed as grams relaxation before and after propranolol, in 5 animals, mean $\pm$ 1 SE. For isoproterenol, a significant reduction occurred ($P < 0.02$) but lidocaine acted in the presence of this β-blocking drug ($P = 0.2$).

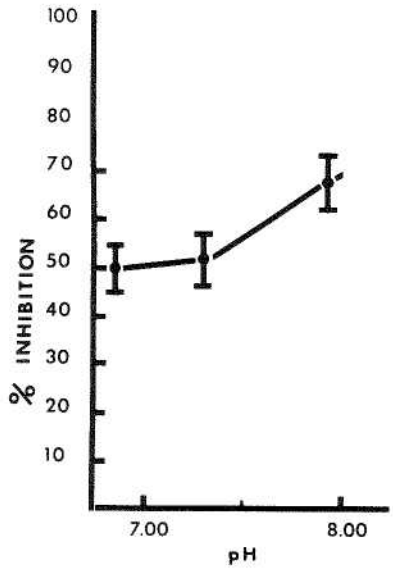

Fig. 7. Effect of pH. The per cent inhibition by lidocaine (0.50 mg per ml) to 8.0-μg bolus doses of histamine at pH 6.71, pH 7.32, and pH 7.90. Response to histamine before lidocaine antagonism showed no significant difference ($P > 0.05$) in the observed pH range. The reduction in hydrogen ion concentration significantly increased ($P < 0.05$) the effectiveness of lidocaine.

on lidocaine can be derived from data in peripheral nerve fibers. Local anesthetics exist both as a free base (B) and as a charged cation (BH^+), with the relative amount of the 2 forms depending on its pKa and the pH of the medium. A decrease in the hydrogen ion concentration will shift the equilibrium toward the presence of free base ($BH^+ \rightleftharpoons B + H^+$). It is the free base that exhibits a greater rate of penetration through the nerve sheath, whereas the charged cation, reformed at the membrane after diffusion, is responsible for the local anesthetic potency in reference to these neural structures (1, 2, 20). Accordingly, the increase in effectiveness of lidocaine in an alkaline solution in our studies indicates that the base form is also important for penetration into the muscle cell of guinea pig trachea. This effect has been described for vascular smooth muscle by Hudgins and Putney (21).

The effectiveness of isoproterenol, a standard β-adrenergic receptor stimulator, was significantly reduced in the presence of propranolol, yet the effectiveness of lidocaine was unaltered. Thus, the apparent mode of action of lidocaine in guinea pig trachealis muscle does not involve β-adrenergic receptor stimulation.

Our results also indicate that lidocaine is effective in protecting against contractions produced by membrane depolarization with square wave electrical stimulation and hypertonic potassium. To these stimuli, lidocaine was effective in the same dose range that was found for histamine and acetylcholine. Hudgins and Weiss (22) have shown in their studies on rabbit aorta that potassium and histamine, through different mechanisms, interact with calcium to induce contraction. Thus, because lidocaine in the same dose range is equally effective toward all studied agonists, its mode of action may be a pathway common to all.

These studies indicate an *in vitro* stabilizing action of lidocaine largely operating upon the smooth muscle cell. The evidence for the latter is surmised from the drug's persistent effect despite application of cholinergic and β-adrenergic blocking drugs, the protection offered during direct electrical stimuli and from histamine-induced muscle contraction. However, a combination of effects or modulation may occur *in vivo*, and translation of this effect to the intact animal is not possible. Furthermore, endoanesthetic effects on pulmonary stretch receptors or other afferent sensory pathways may occur *in situ* by local anesthesia of respective nerve. A small number of studies have dealt with intact guinea pigs. It appears that substantial doses of procaine (40 to 80 mg per kg, administered intraperitoneally) are required against standard agonists, whereas in narcotized dogs similar doses admin-

istered intravenously are spasmolytic but not effective prophylactically (4).

Thus, one local anesthetic of the aminoacylamine class, lidocaine, exhibits nonspecific, noncompetitive, muscle-stabilizing properties *in vitro* under a variety of stimuli: chemical, hypertonic potassium depolarization, and electrical stimulation. Under our limited experimental conditions, its action persists despite cholinergic and β-adrenergic blockade. The mechanism of action of lidocaine in trachealis smooth muscle may include stabilization of the plasma membrane or cellular organules, or an influence on excitation-contraction coupling, or the actual contractile process possibly related to calcium uptake, fluxes, binding, or activity (10, 11; Unpublished data). Such pathway (s) suggested by lidocaine action may serve as a model in guinea pig bronchospasm, or even human asthma, as a complementary approach to treatment.

References

1. Covino, B. G.: Local anesthesia, N Engl J Med, 1972, *286*, 975.
2. Ritchie, J. M., Ritchie, B., and Greengard, P.: On the mode of action of local anesthetics, Am Rev Pharmacol, 1966, *6*, 405.
3. Aceves, J., and Machne, X.: The action of calcium and of local anesthetics on nerve cells and their interaction during excitation, J Pharmacol Exp Ther, 1963, *140*, 138.
4. Lechat, P.: International Encyclopedia of Local Anesthetics, vol. 1, Pergamon Press, New York, 1971.
5. Wiedling, S.: Xylocaine, The Pharmacological Basis of Its Clinical Use, Almquist & Wiksell, Stockholm, 1964.
6. Fleisch, J. H., and Titus, E.: Effect of local anesthetics on pharmacologic receptor systems of smooth muscle, J Pharmacol Exp Ther, 1973, *186*, 44.
7. Furchgott, R. F., and Kirpekar, S. M.: Actions and interactions of norepinephrine, thyamine and cocaine on aorta strips of rabbit and left atria of guinea pig and cat, J Pharmacol Exp Ther, 1963, *142*, 39.
8. Carvalho, A. P.: Calcium-binding properties of sarcoplasmic reticulum as influenced by ATP, caffeine, quinine, and local anesthetics, J Gen Physiol, 1968, *52*, 622.
9. Inoue, F., and Frank, G. B.: Action of procaine on frog skeletal muscle, J Pharmacol Exp Ther, 1962, *136*, 190.
10. Feinstein, M. B., and Paimre, M.: Pharmacological action of local anesthetics on excitation-contraction coupling in striated and smooth muscle, Fed Proc, 1969, *28*, 1643.
11. Feinstein, M. B.: Inhibition of contraction and calcium exchangeability in rat uterus by local anesthetics, J Pharmacol Exp Ther, 1966, *152*, 516.
12. Feinstein, M. B., and Paimre, M.: Mode of anticholinergic action of local anesthetics, Nature, 1967, *214*, 151.
13. Aberg, G., and Andersson, R.: Studies on mechanical actions of mepivacaine (carbocaine) and its optically active isomers on isolated smooth muscle: Role of Ca^{++} and cyclic AMP, Acta Pharmacol Toxicol (Kbh), 1972, *31*, 321.
14. Sinha, Y. K.: Studies on local anesthetic drugs, J Pharm Pharmacol, 1953, *5*, 620.
15. Constantine, J. W.: The spirally cut tracheal strip preparation, J Pharm Pharmacol, 1965, *17*, 384.
16. Ushinski, S. C., Mansmann, H. C., Jr., and Roberts, J.: The influence of initial tension on the reactivity of guinea-pig tracheal preparations to histamine, Arch Int Pharmacodyn Ther, 1971, *190*, 61.
17. Long, J. P., and Chiou, C. Y.: Pharmacological testing methods for drugs acting on the peripheral nervous system, J Pharm Sci, 1970, *59*, 133.
18. Zar, J. H.: Biostatistical Analysis, Prentice-Hall, Inc., Englewood Cliffs, N. J., 1974.
19. Nishimura, N., Morioka, T., Sato, S., and Kuba, T.: Effects of local anesthetic agents on the peripheral vascular system, Anesth Analg (Cleve), 1965, *44*, 135.
20. Narahashi, T., Frazier, D. T., and Yamada, M.: The site of action and active form of local anesthetics. I. Theory and experiments with tertiary compounds, J Pharmacol Exp Ther, 1970, *171*, 32.
21. Hudgins, P. M., and Putney, J. W.: Distribution of local anesthetics and the intracellular pH in vascular smooth muscle, J Pharmacol Exp Ther, 1972, *181*, 538.
22. Hudgins, P. M., and Weiss, G. B.: Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine, and potassium, J Pharmacol Exp Ther, 1968, *159*, 91.