The Effect of a Local Anesthetic, Lidocaine, on Guinea Pig Trachealis Muscle In Vitro

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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 non-specific, 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
contraction and reversal from agonist-induced con-

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 mM; KCl, 4.7 mM; NaHCO₃, 24.8 mM; MgSO₄, 7 H₂O, 2.4 mM; KH₂PO₄, 1.2 mM; CaCl₂, 2.5 mM; glucose, 10.0 mM 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 O₂ and 5 per cent CO₂. PO₂ and P CO₂, and pH of the superfusate were monitored daily with a 313 Blood Gas Analyzer (Instrumentation Laboratories, Inc.). Partial pressures for CO₂ ranged from 35 to 45 mm Hg and approximately 400 mm Hg for O₂ with an average pH of 7.45. Superfuse flow of 5.0 ml per min was maintained by Buchler polystatic pump from a 2-liter reservoir of Krebs Henseleit.

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 mM 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 potassium chloride concentration. Lidocaine, 0.25 µg 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 mM; these were applied for 60 sec. Equiopotent 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 grains 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 contraction. 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 µg 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. Supernusate 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 linearity.

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...
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 hyp-

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 contractions 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.

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 Kd (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 the 3 selected potassium concentrations (P < 0.05) (figure 5B). The relative percent of inhibition at a given K⁺ concentration was constant (50 percent at 40 mM).

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
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 ED₅₀ 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 ED₅₀, being most potent against histamine (ED₅₀ = 0.01 µg) and least potent against hypertonic potassium (ED₅₀ = 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 ± 1 SE. For isoproterenol, a significant reduction occurred ($P < 0.02$) but lidocaine acted in the presence of this $\beta$-blocking drug ($P = 0.2$).

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 $pK_a$ 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 $\beta$-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 $\beta$-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 \textit{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 $\beta$-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 \textit{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 \textit{in vivo} 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 spasmylytic but not effective prophylactically (4).

Thus, one local anesthetic of the aminoacrylamine class, lidocaine, exhibits nonspecific, non-competitive, 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 organelles, 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
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.