THE EFFECTS OF A LOCAL ANESTHETIC (BUPIVACAINE) ON THE ELECTRICAL PROPERTIES OF THE SKELETAL MUSCLE CELL MEMBRANE

by

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INTRODUCTION

Several theories regarding the mechanism by which local anesthetics cause a decrease in cellular excitability have been postulated and the subject has been extensively reviewed (Watson, 1960; Wiedling and Tegner, 1963; Ritchie and Greengard, 1966; Narahashi and Moore, 1968; deJong, 1970). Three of the more prominent mechanisms proposed are 1) membrane stabilization, 2) control of ionic "gates", and 3) receptor inhibition.

Local anesthetics may act as non-specific membrane stabilizers, reducing the permeability of the membrane to ions (Taylor, 1959) by an increase in surface pressure (Shanes and Gershfeld, 1960; Skou, 1961) which could cause closing of "pores" in the membrane or alter the amount of folding or unfolding of large surface molecules, hence altering their biological activity. Thus, physical changes produced by penetration of the drug into the membrane might be responsible for the reduction in ionic conductances seen in nerve (Shanes et al., 1959; Taylor, 1959; Aceves and Machne, 1963; Goldman and Blaustein, 1966; Narahashi et al., 1967; Narahashi and Moore, 1968) and muscle fibers (Inoue and Frank, 1962; Maeno, 1966) exposed to local anesthetics.

A second hypothesis, using evidence from axon perfusion

experiments, suggests that local anesthetics may act on the internal as well as the external surface of the membrane to block electrical activity by controlling the "gates" responsible for conductance changes during excitation (Narahashi et al., 1967; Narahashi and Frazier, 1968; Narahashi and Moore, 1968). Control of ion fluxes might be achieved by blocking specific ionic channels (Goldman and Blaustein, 1966; Narahashi et al., 1967) or by binding of the drug to reactive groups on the membrane necessary for excitation (Feinstein, 1964; Blaustein and Goldman, 1966). The high lipid solubility (Shanes and Gershfeld, 1960; Skou, 1961; Cuthbert, 1967) of local anesthetics would make sites on either surface of the membrane equally accessible even if the compound were applied externally (Narahashi et al., 1967).

A third theory concerns the effect of local anesthetics on the acetylcholine receptor protein at the neuromuscular junction, where they act as receptor inhibitors capable of preventing or reversing the depolarization caused by acetylcholine (Higman and Bartels, 1962).

The current study was undertaken to ascertain whether the changes in cellular excitability produced by a local anesthetic, bupivacaine[1] (dl-1-butylpipecoloxylidide hydrochloride), could be correlated with changes in the electrical properties of the muscle cell membrane. Bupivacaine was used as a representative local anesthetic of greater potency than

1. Marcaine^{R}, courtesy of Sterling-Winthrop Research Institute.

some of the more common members of this class of drugs (Albert and Lofstrom, 1965). The isolated rat diaphragm was chosen for the study because of ease of preparation and ready access to muscle fibers for direct microelectrode penetration.

METHODS

Male or female Sprague-Dawley rats, 260-470 g, were anesthetized with ether and their diaphragms excised intact. The muscle was bisected dorso-ventrally at the central tendon and secured, abdominal side up, in a petri dish lined with Sylgard[2] encapsulation resin. The hemidiaphragm was bathed in either normal oxygenated rat Krebs' solution (NaCl, 146 mM; KCl, 4.0 mM; $NaHCO_3$, 11.9 mM; KH_2PO_4, 1.0 mM; $MgCl_2$, 1.0 mM; $CaCl_2$, 2.0 mM; glucose, 11.0 mM) or oxygenated rat Krebs' containing 0.0005%, 0.005%, 0.05%, or 0.1% bupivacaine. The pH of these solutions was 7.6-7.7 with the exception of the 0.1% concentration, where pH dropped to 6.7. It is likely that a pH shift of this magnitude would cause a disproportionate increase in the amount of bupivacaine present in the cationic form relative to the amount present as free base. Since the cationic form of the molecule may be the more potent one in blocking electrical activity of membranes (Ritchie and Greengard, 1961; Dettbarn, 1962), the actual potency of 0.1% bupivacaine at pH 6.7 may be greater than indicated by its concentration. While an increase in hydrogen ion concentration alone will cause

2. Dow Corning Corporation

diminution of sodium conductance, the effect does not become apparent until pH drops below 6 (Hille, 1968; Sokoll and Thesleff, 1968). Experiments were done at 21-23° C. Fifteen minutes were allowed for equilibration between bathing medium and muscle before measurements were begun. With the exception of resting potential, which exhibited a slow decline at 0.1% bupivacaine, measurements made on fibers bathed in normal rat Krebs' solution and on bupivacaine-treated fibers showed no variation that could be ascribed to the length of time that the muscle remained in vitro.

Action potential measurements. The ability of muscle fibers to produce action potentials upon direct electrical stimulation and the configuration of these potentials were determined for fibers bathed in normal rat Krebs' solution and 0.005%, 0.05%, and 0.1% bupivacaine-rat Krebs' solution. Three hemidiaphragms from two rats were used for this series. Each fiber was impaled with two 3M KCl-filled, glass microelectrodes (15-25 MΩ resistance), the tips of which were 30-70 μ apart. One electrode was used to stimulate the fiber with depolarizing current pulses of progressively increasing strengths derived from a stimulator and stimulus isolation unit (Grass S4 and SIU). The magnitude of the current pulse was determined by measuring the voltage drop across a 200 KΩ resistor placed in series with the electrode and was displayed on one beam of a dual beam oscilloscope (Tektronix 502). The transmembrane potential change pro-

duced by the passage of the current pulse through the membrane was detected by the second electrode and displayed as the second oscilloscope trace. Photographic records of these traces were taken (Grass C4 camera) and measurements of threshold, action potential amplitude, and the amount of depolarization necessary to produce an action potential (depolarization to threshold) were made. Threshold was defined as the point at which the response of the membrane became independent of the applied stimulus (cf. fig. 2) and was found by extrapolation of the linear segments of the initial, non-propagating electrotonic portion of the action potential and the rising phase of the regenerative potential. Action potential amplitude was the absolute magnitude (in mV) of the difference between resting potential and the maximum transmembrane potential attained during the action potential. Depolarization to threshold was the absolute magnitude (in mV) of the difference between resting potential and threshold. Observations were made on each hemidiaphragm in normal rat Krebs' solution to be certain the fibers were undamaged and capable of producing normal action potentials before any drug was added. This solution was then replaced with one containing 0.005%, 0.05%, or 0.1% bupivacaine and, after a 15 minute equilibration period, attempts to generate action potentials were repeated. Records were taken on each muscle over a one and one-half hour period.

Resistance and length constant measurements. Measurements of input resistance(V/I), transverse resistance of a unit

length of membrane (r_m), internal longitudinal resistance per unit length of fiber (r_i), and fiber length constant (λ) were obtained by the method of square pulse analysis (Hodgkin and Rushton, 1946) from 16 rat hemidiaphragms bathed in normal rat Krebs' solution and 0.0005%, 0.005%, 0.05%, and 0.1% bupivacaine-rat Krebs' solution. Since the transmembrane potential changes of both control and drug-treated fibers to hyperpolarizing currents were linear over the current range used in these experiments (fig. 1), it was assumed that the cable equation could be used to calculate resistances and the length constant. The cable equation states that when a steady, hyperpolarizing current pulse (I) is passed through the membrane, the potential change (V) across the membrane is given by the equation $V=\frac{1}{2}I\sqrt{r_m\ r_i}\ \exp(-x/\sqrt{r_m/\ r_i}$ -- where x is interelectrode distance; r_m the transverse resistance of a unit length of membrane; r_i the internal longitudinal resistance per unit length of fiber; $\sqrt{r_m/\ r_i}$ is λ, the length constant; and $\frac{1}{2}\sqrt{r_m\ r_i}$ is V/I, the input resistance.

Each fiber was impaled with two microelectrodes, one for passing rectangular, hyperpolarizing current pulses (I) through the membrane and one for recording the resulting transmembrane potential changes (V). Records were obtained with interelectrode intervals of 45, 300, and 600 μ. Values of V/I were then plotted on semilog$_{10}$ paper against the interelectrode distance and the line was extrapolated to the y-axis to give the input resistance. If the plot was not linear it was assumed that the fiber was damaged and the data were dis-

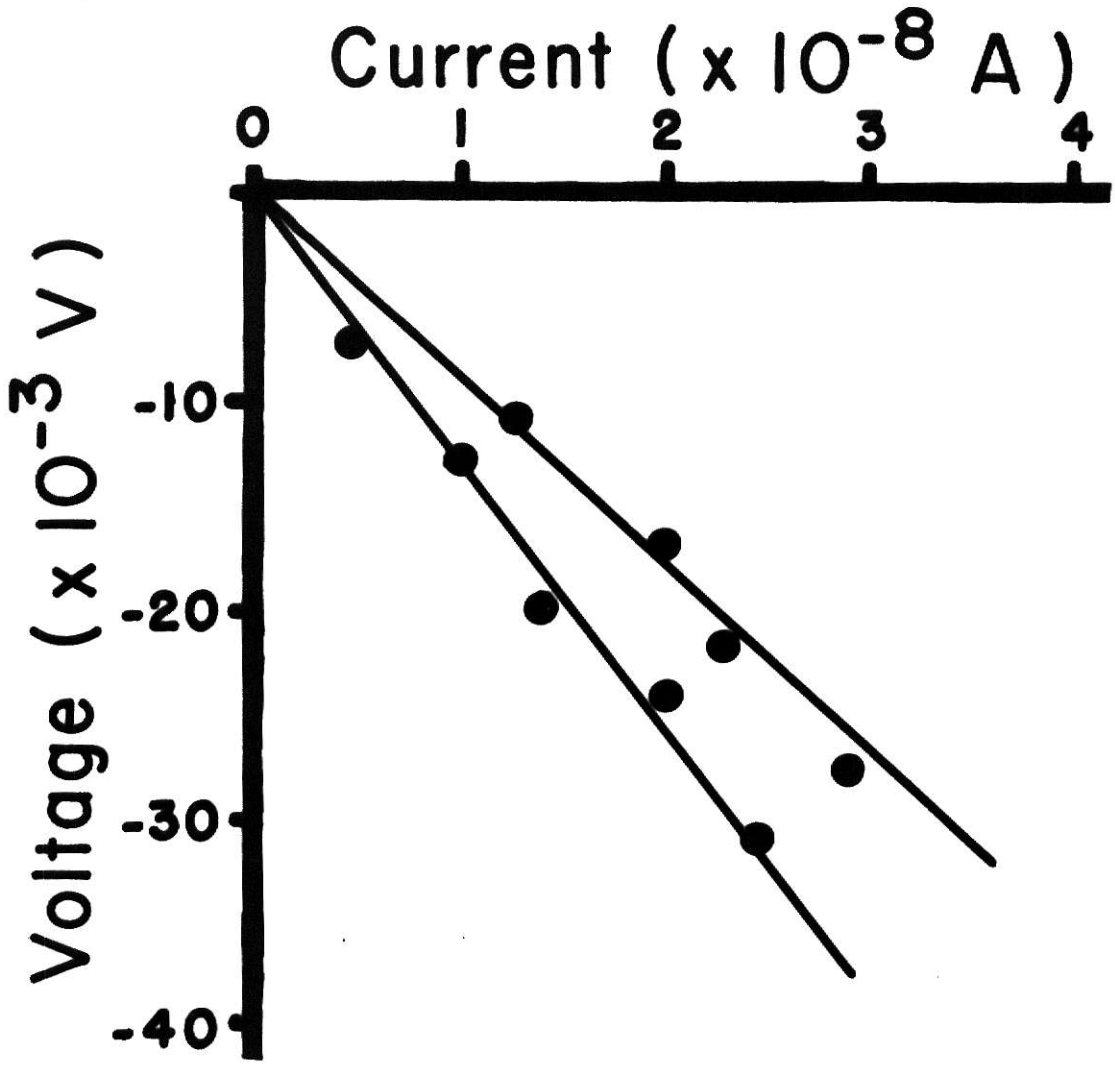

Figure 1. Current-voltage relationships for two muscle fibers from the rat diaphragm. Top line is from a fiber bathed in 0.005% bupivacaine, bottom line is from a control fiber. The relationship was linear for fibers at all concentrations of bupivacaine used.

carded. The slope of the line equals $-1/(2.303\ \lambda)$. From λ the values of the intercept and the slope, r_m and r_i can be calculated. For undamaged, non-drug-treated fibers values of r_m and r_i can be used to obtain an estimate of fiber radius, transverse resistance of a unit area of membrane, and membrane capacitance. However, local anesthetics are known to be highly lipid-soluble (Skou, 1961; Cuthbert, 1967), and if bupivacaine were incorporated into the lipid portion of the membrane the resulting lipid-anesthetic "solution" might have, from a purely physical-chemical standpoint, a different electrical resistance than the lipid alone (Tasaki, 1955; Feinstein, 1964). If membrane resistance was changed by this process, the calculation of fiber radius and subsequent values which depend on radius would be in error. For this reason only λ, V/I, r_m, and r_i will be compared here.

Statistical analysis. A one-way analysis of variance (Snedecor and Cochran, 1970) was used to test for differences between treatment means. When a significant F value was obtained the Duncan Multiple Range test (Snedecor and Cochran, 1970) as modified by Kramer (1956) was used to determine which means differed from each other.

RESULTS

Action potential measurements. As the concentration of bupivacaine in the bathing medium was increased the muscle fibers exhibited a decreased ability to produce action potentials (fig. 2). Figure 2A illustrates a response from a control fiber, which had an amplitude of 117 mV and a threshold level of -41 mV. Figures 2B and 2C were obtained from fibers exposed to 0.005% bupivacaine. A decrease in amplitude of the action potential to 110 mV and 72 mV, respectively, was observed, while threshold increased to -35 and -36 mV, respectively. The magnitude of the depolarization to threshold increased from 34 mV for the fiber shown in fig. 2A to 43 and 38 mV in the fibers exposed to this concentration of drug. Figures 2D and 2E are tracings from fibers bathed in 0.05% bupivacaine and show similar but slightly more pronounced alterations. Action potential amplitudes were 97 and 81 mV, respectively; threshold levels were -35 and -26 mV, respectively; and the magnitudes of the depolarization to threshold were 40 and 46 mV, respectively. Figure 2F represents the response of a fiber exposed to 0.1% bupivacaine. At this concentration the fiber was incapable of producing an action potential regardless of the magnitude of the applied stimulus. Thus, along with the decreased amplitude of action potentials which occurred as the bupiva-

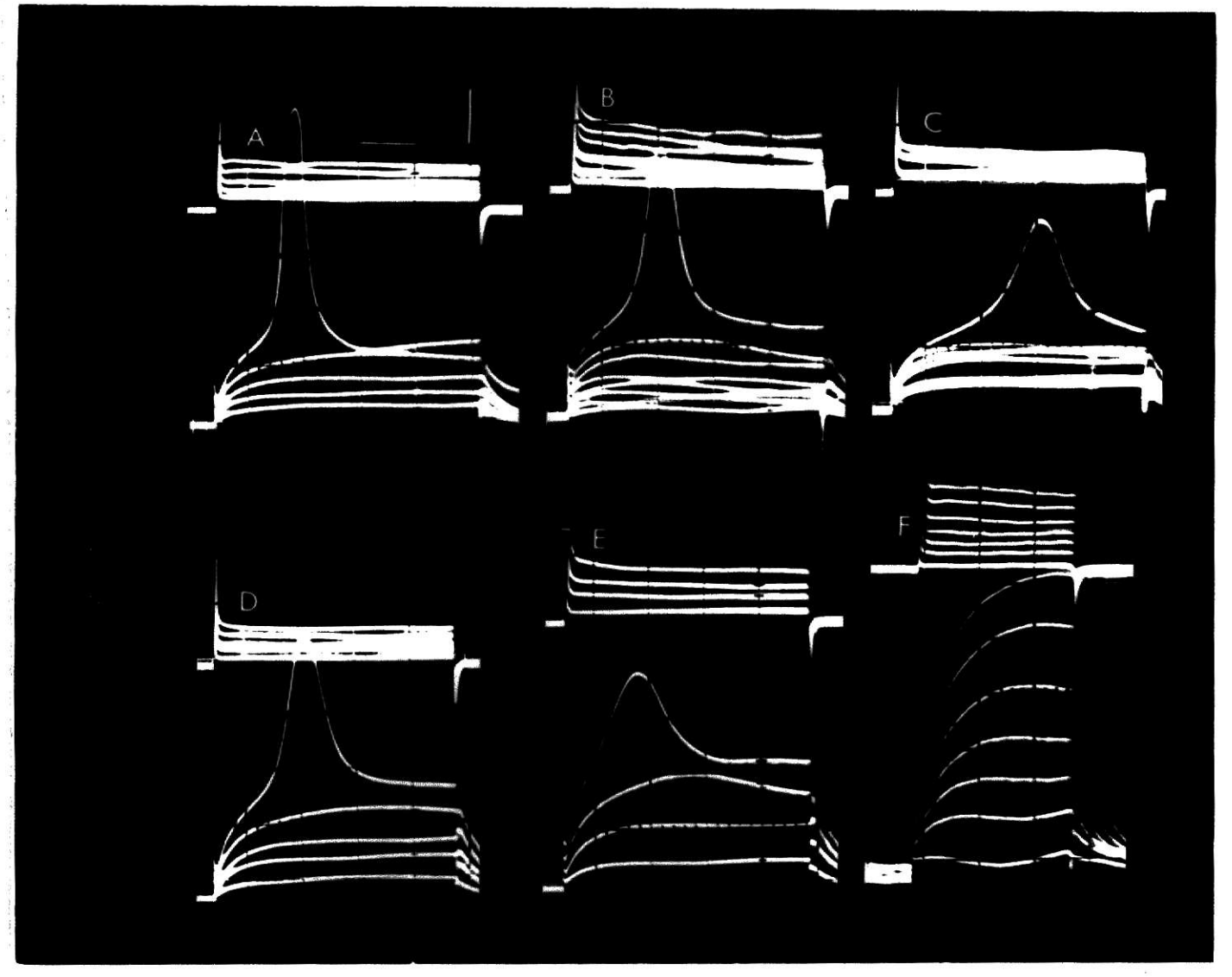

Figure 2. Intracellular recordings of action potentials from rat diaphragm muscle fibers. Top trace is the current passed through the membrane, lower trace is the transmembrane potential change of the fiber. Vertical line above A is 50 nanoamps for the current trace and 20 mV for the voltage trace; horizontal line is 5 msec. A, Control; B and C, 0.005% bupivacaine; D and E, 0.05% bupivacaine; F, 0.1% bupivacaine.

caine concentration was increased, there was also an increase in the number of muscle fibers which could not generate an action potential at all (fig. 3).

A summary of the data from all fibers examined is presented in table 1. Concentrations of bupivacaine ranging from 0.005% to 0.1% had no effect on resting potential during the recording period. There was, however, a concentration-dependent reduction in the ability of muscle fibers to produce action potentials. While all 25 control fibers were capable of producing action potentials, only 15 of 22 fibers exposed to 0.005% bupivacaine were capable of generating an all-or-none response. Increasing the concentration of bupivacaine to 0.05% resulted in only 10 of 18 fibers being able to produce an action potential. When this concentration was doubled (to 0.1%) all 28 fibers tested were incapable of generating an action potential. Action potential amplitudes decreased with increasing concentration of drug (table 1) until at 0.1% bupivacaine no action potentials could be generated. Threshold levels also increased as the concentration of bupivacaine was increased. Since resting potentials were unaffected by the drug in this set of experiments, the amount of depolarization required to reach threshold was likewise increased. These data suggest that bupivacaine affected the excitability of skeletal muscle fibers in a manner dependent upon the concentration of the drug. Increased drug concentration resulted in decreased excitability.

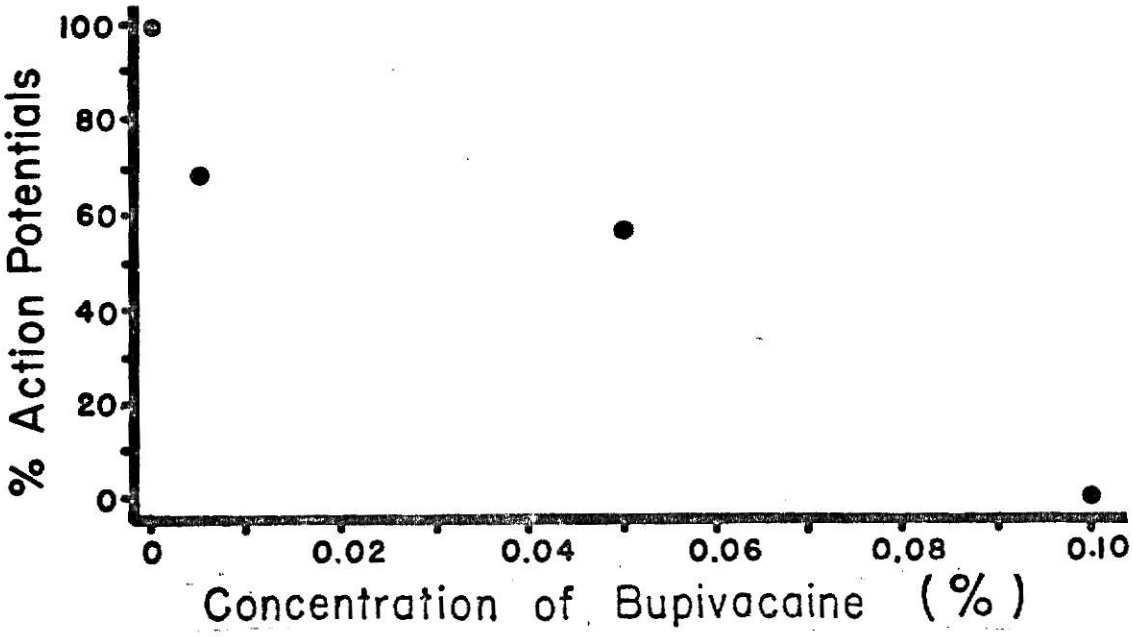

Figure 3. Percentage of action potentials obtained at each concentration of bupivacaine.

Resistance and length constant measurements. To correlate the effects of bupivacaine on the action potential with possible changes in membrane properties, determinations of resistances and length constant were done for untreated fibers and fibers bathed in 0.0005%, 0.005%, 0.05%, and 0.1% bupivacaine-rat Krebs' solution. Examples of the magnitudes of transmembrane potential changes obtained when current was passed through the membrane are shown in figure 4. As the interelectrode distance was increased, the magnitude of the electrotonic potential change across the membrane decreased as predicted by the cable equation. A plot of semilog$_{10}$ V/I vs interelectrode distance (fig.5) was linear and provided the basis for the calculation of λ, r_m, and r_i.

Table 2 summarizes the data for all fibers examined in this series of experiments. As indicated in table 1, concentrations of bupivacaine less than 0.1% had no effect on the resting potential of the fibers. However, 0.1% bupivacaine caused the fibers to depolarize slowly, resulting in a significant reduction in the mean value. In this series of experiments the muscle fibers were exposed to the drug almost four times as long as those used for action potential measurements, and the loss of resting potential was seen to increase with time.

While all four concentrations of bupivacaine produced an increase in input resistance (V/I) over the control value, the effect at 0.0005% did not differ significantly from that at 0.005%, 0.05%, or 0.1% bupivacaine. The rise in

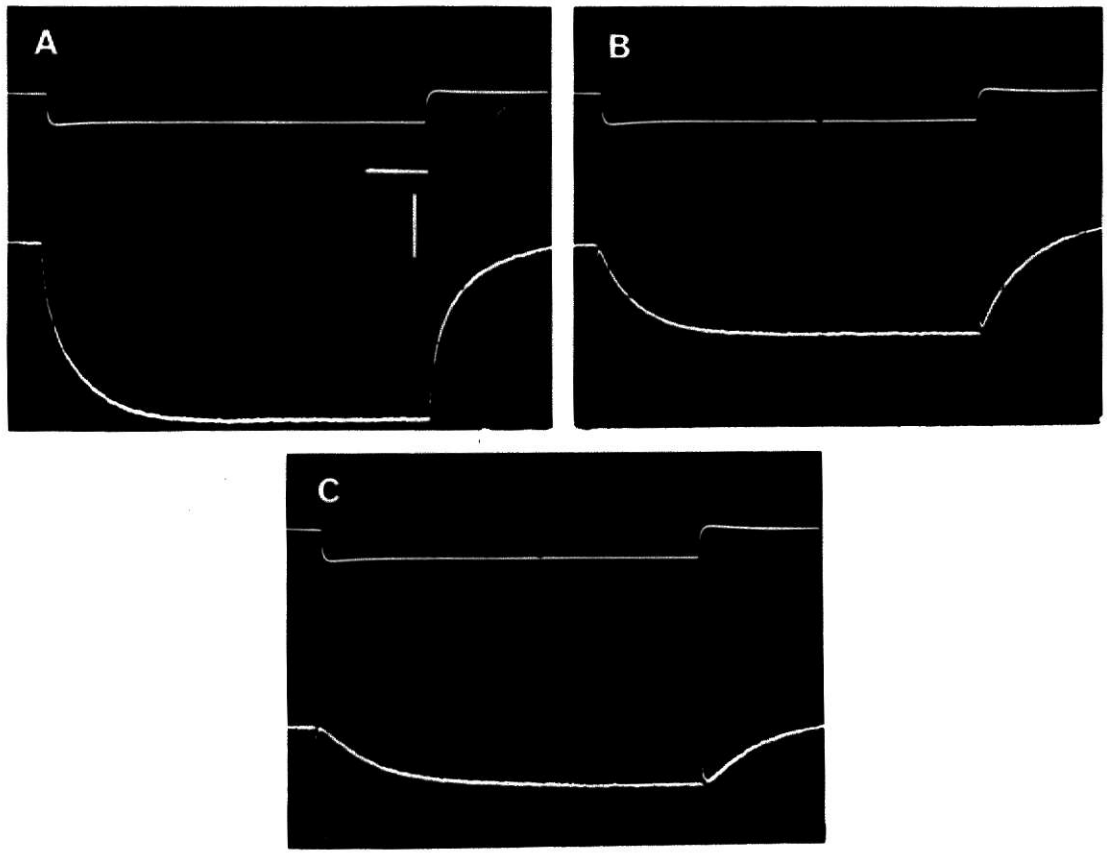

Figure 4. Intracellular recordings from a rat diaphragm muscle fiber bathed in normal rat Krebs' solution. Top trace is the current passed through the membrane, lower trace is the resulting transmembrane potential change of the fiber. Vertical line in A is 50 nanoamps for the current trace, 5 mV for the voltage trace; horizontal line is 2 msec. The resting potential of this fiber was -73 mV. Interelectrode distance: A, 0.045 mm; B, 0.30 mm; C, 0.60 mm.

Table 1. The effects of bupivacaine on the muscle action potential.

Treatment	Total No. of Fibers	RP[a,b] (mV)	N[c] (AP)	Action Potential Amplitude[a] (mV)	Threshold[a] (mV)	Depolarization to Threshold[a] (mV)
Control	25	77 ± 6[1]	25	102 ± 5[1]	-46 ± 6[1]	31 ± 4[1]
0.005% bupivacaine	22	75 ± 3[1]	15	90 ± 3[2]	-38 ± 6[2]	37 ± 5[2]
0.05% bupivacaine	18	74 ± 3[1]	10	86 ± 9[3]	-34 ± 4[3]	41 ± 4[3]
0.1% bupivacaine	28	75 ± 6[1]	0	-------	--------	--------

a. Mean ± SD

b. Resting potential

c. Number of fibers giving action potentials.

1, 2, 3. Like superscripts denote means that are not significantly different. Unlike superscripts denote means that are significantly different at $P < 0.05$.

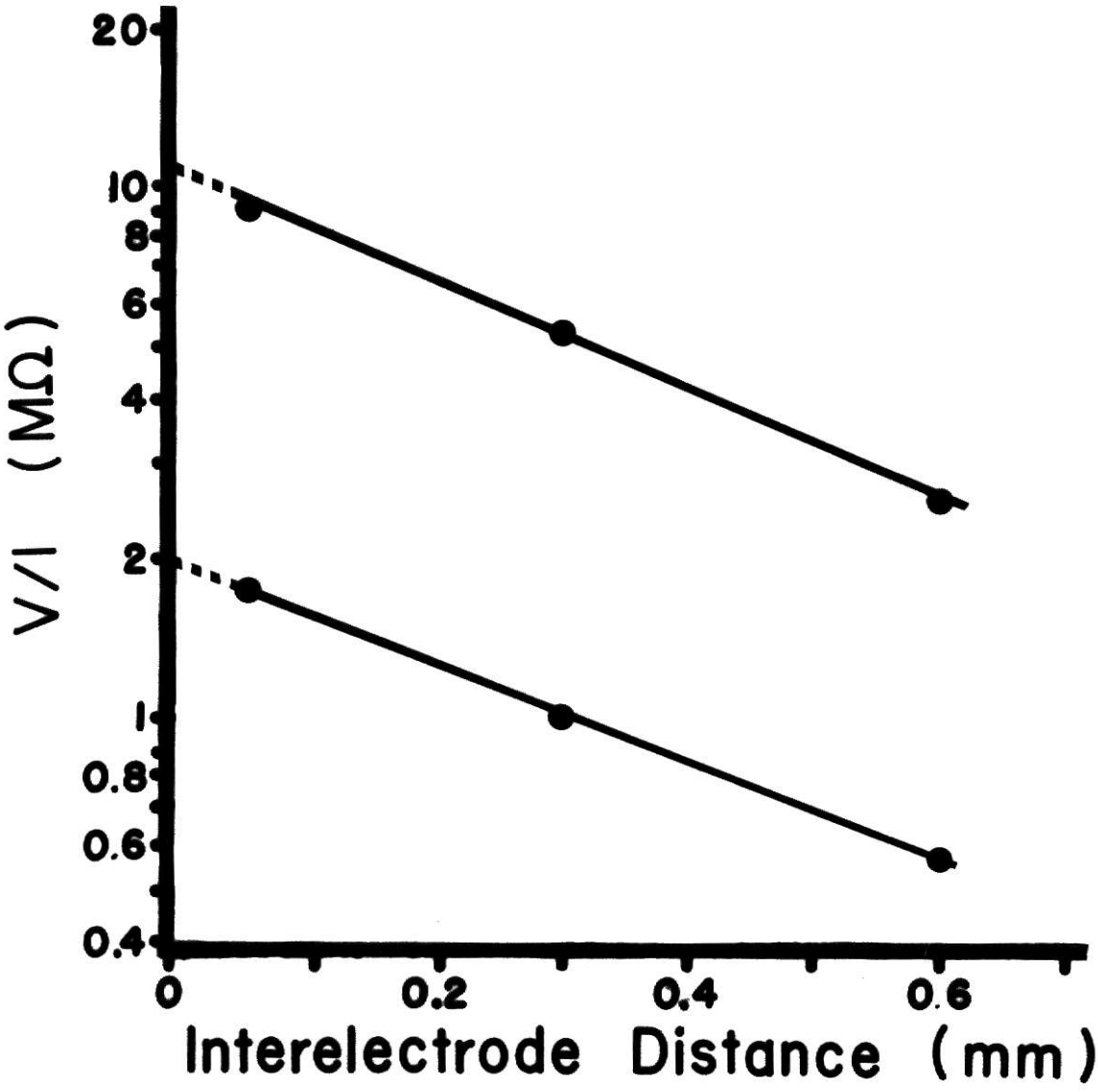

Figure 5. The relationship of V/I to interelectrode distance for two fibers. Top line is from a fiber treated with 0.1% bupivacaine, bottom line is from a control fiber. V/I is seen to be greater for the bupivacaine-treated fiber than for the control, while the slopes of the lines remain similar.

Table 2. The effects of bupivacaine on membrane resistance and length constants.

Treatment	No. of Muscles	No. of Fibers	RP[a,b] (mV)	V/I[a] (MΩ)	r_m[a] (x 10^5 Ω cm)	r_i[a] (x 10^7 Ω/cm)	λ (mm)
Control	5	63	73 ± 5[1]	0.40 ± 0.18[1]	0.37 ± 0.16[1]	1.85 ± 1.11[1]	0.47 ± 0.13[1]
0.0005% bupivacaine	2	44	74 ± 7[1]	0.58 ± 0.27[2]	0.52 ± 0.25[2]	2.64 ± 1.34[2]	0.46 ± 0.06[1]
0.005% bupivacaine	3	50	73 ± 6[1]	0.60 ± 0.25[2]	0.60 ± 0.24[2]	2.48 ± 1.43[2]	0.51 ± 0.10[2]
0.05% bupivacaine	4	47	74 ± 7[1]	0.54 ± 0.24[2]	0.50 ± 0.22[2]	2.38 ± 1.36[2]	0.48 ± 0.10[1]
0.1% bupivacaine	2	43	56 ± 8[2]	0.68 ± 0.26[2]	0.57 ± 0.20[2]	3.33 ± 1.47[3]	0.43 ± 0.06[3]

a. Mean ± SD

b. Resting potential

1, 2, 3. Like superscripts denote means that are not significantly different. Unlike superscripts denote means that are significantly different at $P < 0.05$.

V/I varied from 35-70% of the control value, with no clear dose-response relationship being evident. Changes in transverse resistance of a unit length of membrane (r_m) followed the same pattern as input resistance values, with increases of 35-62% of the control value. Internal longitudinal resistance per unit length of fiber (r_i) increased by 29-43% over a concentration range of 0.0005%-0.05% bupivacaine, with no significant variations within this range. At 0.1% bupivacaine, however, a further significant increase, to 80% above the control value, was seen. These results indicate that bupivacaine produces an increase in each of the three measured resistance parameters of the muscle fiber, but that these changes are not dependent upon drug concentration.

Length constant measurements also failed to reveal any simple dose-response relationship. A slight (9%) but significant increase in λ over that of the control value was seen at 0.005% bupivacaine but the value at 0.05% was not significantly different from the control value. At 0.1% bupivacaine λ dropped to 89% of the control value.

DISCUSSION

Bupivacaine appears to affect the excitability of the skeletal muscle fiber in much the same way that other local anesthetics affect nerve and muscle cells. A decrease in action potential amplitude (Shanes et al., 1959; Inoue and Frank, 1962; Aceves and Machne, 1963; Goldman and Blaustein, 1966; Narahashi et al., 1967), increase in threshold (Shanes,et al., 1959; Inoue and Frank, 1962; Blaustein and Goldman, 1966; Goldman and Blaustein, 1966), and increase in depolarization to threshold (Inoue and Frank, 1962) are all characteristic of the action of local anesthetics in general and were also observed in muscle fibers treated with bupivacaine.

The above observations, together with direct measurements of ionic currents in both nerve (Shanes et al., 1959; Taylor, 1959; Aceves and Machne, 1963; Goldman and Blaustein, 1966; Narahashi et al., 1967; Narahashi and Moore, 1968) and skeletal muscle cells (Inoue and Frank, 1962; Maeno, 1966) have shown that local anesthetics interfere with the mechanism by which the rapid rise in sodium conductance is effected during excitation. The increase in sodium conductance is both delayed in onset and reduced in magnitude by these drugs, so that the inward sodium current is not only smaller

than normal but is also more effectively counterbalanced by the outward potassium current. The results of these conductance changes are manifested by increased threshold levels and decreased amplitude and rate of rise of the action potential.

The mechanism by which local anesthetics cause the above changes is not known. The initial supposition leading to the current study was that local anesthetics might cause an increase in membrane resistance that would closely parallel the observed decrease in cellular excitability. A resistance increase, perhaps caused by complex molecular interactions between membrane constituents and the drug (Taylor, 1959; Skou, 1961; Feinstein, 1964; Blaustein and Goldman, 1966; Goldman and Blaustein, 1966), might result in the characteristic conductance changes by restricting ion movement through the membrane. If this were the case, one would expect resistance to increase as cellular excitability decreased in a dose-related manner. Blocking of action potential generation would occur when membrane resistance reached a sufficiently high level to impede current flow to the extent that a regenerative increase in sodium conductance could not occur. Even though bupivacaine did produce an increase in membrane resistance in skeletal muscle fibers, the response was not dose-dependent. Since the proportion of cells in which action potential generation was blocked continued to increase with drug concentration, it appears that the

changes in cellular excitability were not directly related to membrane resistance changes.

Resting potentials were not affected by the drug, which would suggest that resting membrane conductances were not interfered with to any extent by bupivacaine.

It is possible that the membrane resistance increase is merely a function of the presence of another molecular species in the lipid portion of the membrane. Anesthetic molecules dissolved in the membrane lipids may serve only as a readily available reservoir from which the drug could diffuse as needed to active sites on either side of the membrane. Apparently the resistance increase caused by bupivacaine is not of primary importance in the process by which impulse generation is blocked in skeletal muscle fibers.

SUMMARY

A study of the effects of a local anesthetic (bupivacaine) on muscle fiber membranes of the isolated rat diaphragm was conducted to determine whether the decrease in membrane excitability observed in the presence of this anesthetic could be correlated with changes in the electrical properties of the membrane. Exposure to bupivacaine reduced the ability of fibers to produce action potentials. As the drug concentration was increased changes in the configuration of the action potential were seen first, followed by action potential block. The severity of effect was directly dependent upon drug concentration. At 0.1% bupivacaine all fibers were blocked.

Bupivacaine caused increases in input resistance, transverse membrane resistance, and internal longitudinal resistance of the muscle fibers as determined by the method of square pulse analysis. These effects were not dependent upon drug concentration. Input resistance and transverse membrane resistance reached plateau values at 0.0005% bupivacaine. Internal longitudinal resistance behaved similarly, except for a slight further increase at 0.1% bupivacaine. Resting potentials were unaffected by the drug, except for fibers exposed to 0.1% bupivacaine for prolonged periods. At this concentration resting potentials decreased gradually with

increased length of exposure to the drug.

Because the increase in membrane resistance bore no direct relationship to the decrease in cellular excitability the data suggest that an increase in membrane resistance is not a primary factor in the loss of excitability observed in muscle fibers treated with bupivacaine.

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AN ABSTRACT

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The effects of a local anesthetic (bupivacaine) on the skeletal muscle fiber membrane were studied using the isolated rat diaphragm preparation. The objective of the study was to determine if the decrease in cellular excitability produced by this local anesthetic could be correlated with changes in the electrical properties of the muscle cell membrane.

Intracellular microelectrodes were used for direct electrical stimulation and recording of the resulting transmembrane potential changes from muscle fibers exposed to concentrations of 0.005%, 0.05%, and 0.1% bupivacaine-rat Krebs' solution. Comparison of the configuation of the action potentials from drug-treated fibers with action potentials from fibers bathed in normal rat Krebs' solution showed that bupivacaine, like other local anesthetics, caused a decrease in cellular excitability as the drug concentration was increased. Compared with a control value of 102 mV, action potential amplitude in fibers exposed to 0.005% bupivacaine was reduced to 90 mV. Threshold, which was -46 mV for control fibers, was increased to -38 mV at this concentration of the drug. Since resting potentials were relatively unaffected by the drug, the amount of depolarization necessary to generate an action potential was likewise increased from 31 mV for control fibers to 37 mV

for fibers treated with 0.005% bupivacaine. Similar but more pronounced alterations were observed at larger concentrations of bupivacaine.

Studies of membrane electrical propertie were done by the method of square pulse analysis. Input resistance, transverse resistance of a unit length of membrane, internal longitudinal resistance per unit length of fiber, and the length constant of each fiber were calculated. Concentrations of 0.0005%, 0.005%, 0.05%, and 0.1% bupivacaine were used. Long-duration exposure (2-5 hrs) of muscle fibers to 0.1% bupivacaine caused resting potential to drop from 75 mV for control fibers to 56 mV for fibers exposed to 0.1%. Lower drug concentrations did not affect resting potential. Input resistance increased an average of 50% above the control value over the range 0.0005%-0.1% bupivacaine. Variations within this concentration range were not significant. Transverse resistance followed a similar pattern with an average increase of 48% above the control value. Internal longitudinal resistance increased an average of 35% above the control value over a range of 0.0005%-0.05% bupivacaine. Variations within this range were not significant, but a further significant increase, to 80% above the control value, occurred at 0.1% bupivacaine. The length constant showed a slight increase at 0.005% and a slight decrease at 0.1% bupivacaine.

In the presence of bupivacaine the excitability of the muscle fiber membrane, as measured by the capacity to generate an action potential, was impaired in a manner de-

pendent upon drug concentration. Over the same concentration range bupivacaine also produded increases in membrane resistance, but a dose-dependent relationship was not evident. Resistance increases at the lowest concentration did not vary significantly from those increases observed at the higher concentrations of bupivacaine. Because the increases in membrane resistance had no direct relation to the progressive decrease in excitability caused by the drug the data suggest that an increase in membrane resistance is not a primary factor in the loss of excitability observed in muscle fibers treated with bupivacaine.