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THE EFFECTS OF GENERAL CENTRAL NERVOUS SYSTEM DEPRESSANTS ON THE EXCITABILITY OF SKELETAL MUSCLE

by

© HARPAL S. BUTTAR

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Effects of General Central Nervous System Depressants on the Excitability of Skeletal Muscle" submitted by Harpal S. Buttar in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Evidence has been accumulating in favour of the view that both local and general anesthetics block electrical excitability in a variety of tissues by inhibiting the specific increase of Na^+ conductivity which normally follows depolarization of the membrane. The present investigation was initiated to further test this view using a wide variety of neurotropic compounds, capable of producing CNS depression and transmission block when injected locally. Isolated sartorius muscles of Rana pipiens were used to investigate the effects of chlorpromazine, promethazine, diphenhydramine, scopolamine, meperidine, morphine, gamma-hydroxybutyrate and gamma-butyrolactone on certain features of the active muscle membrane, using extracellular electrode and intracellular microelectrode techniques.

Studies with extracellular electrodes showed that application of all the aforementioned drugs decreased both the amplitude of the compound action potential and the excitability of the muscle fibres. A slow and only partial recovery of the fibres was seen following the treatment with chlorpromazine, promethazine and diphenhydramine. However with rest of the drugs, the depressed responses were reversed to the control level when the preparation was returned to the Ringer's solution.

Experiments with two microelectrodes inserted into the same fibre revealed that the depolarization required to initiate an action potential (threshold depolarization), and the amount of current needed

to produce this depolarization (threshold current) were increased by all the drugs used in this study. Effective membrane resistance, and the membrane time constant (τ_m) were increased after the treatment with promethazine (5×10^{-6} g/ml), diphenhydramine, scopolamine and gamma-hydroxybutyrate (15×10^{-3} g/ml). However, exposure to chlorpromazine, promethazine (1 and 2.5×10^{-6} g/ml), morphine, meperidine, gamma-butyrolactone and low concentrations of gamma-hydroxybutyrate (5 and 10×10^{-3} g/ml) produced no appreciable change in τ_m and in the effective membrane resistance.

The maximum rate of rise and the overshoot of the action potential (used as a measure for inward sodium current) were decreased by all the drugs tested. Both these effects were antagonized by increasing the Na^+ concentration (from 114 to 171 mM) in the extracellular medium. The resting potential remained essentially unchanged in the drug-treated muscles.

The results obtained suggest that a large variety of central depressants block excitability of the frog's sartorius muscle by suppressing the specific increase of Na^+ conductance accompanying the excitation of the membrane. It is suggested that the hypnosis produced by all the drugs used in the present study may involve a mechanism of action similar to that observed in skeletal muscle fibres.

Gamma-aminobutyric acid (GABA) treatment also decreased the excitability of the fibres. Addition of excess Na^+ into the bathing solution did not antagonize the effect of GABA, but rather produced a further depression in the rate of rise and the overshoot of the

spike. All these effects of GABA were duplicated by iso-osmolar concentrations of sucrose. These observations indicate a lack of anesthetic effect of GABA on the frog's sartorius muscle, and suggest that the GABA-induced depression of excitability is most likely due to the hyperosmotic effect of the bathing solution on the fibre membrane.

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TO

My Wife, Harindar

My Daughter, Parneet

and My Son, Harkerat.

CHAPTER I

INTRODUCTION

CHAPTER I. INTRODUCTION

THE IONIC BASIS OF ELECTRICAL ACTIVITY

(A) General Comment:

The ionic, or membrane theory of bioelectricity originated with Bernstein's attempt (1902) to account for the negativity inside muscle and nerve cells relative to their outside. This explanation of the resting potential was based on the then recently developed theory of semi-permeable membranes and is no longer acceptable in total. However, Bernstein also contributed a suggestion which has been amply verified during the past three decades; that the living membranes can change their permeability characteristics when excited by a stimulus. Thus he was able to regard the electrogenic responses of all excitable cells, namely nerves, muscles, sensory and electric organs and glands, as changes of the membrane potential from its resting state. These changes can be depolarizing or hyperpolarizing in sign, but the former, particularly those which result in an all-or-none action potential or spike, are the more prominent and have been studied the most.

The ionic theory has been very much amplified and improved largely through the work of Hodgkin and colleagues (Hodgkin, 1951, 1958). It now provides a remarkable coherent theory which accounts quantitatively for the spike in squid giant axons and to a large degree in several types of nerve and muscle cells.

(B) Ionic Inequalities:

The ionic theory is based on the fact that the electrolyte composition of the extracellular fluid differs markedly from that of its internal milieu (MaCallum, 1905). As for example, the Na^+ concentration inside the frog sartorius muscle is about 15 mM, while in the interstitial fluid it is 110 mM. The K^+ concentration on the other hand is relatively low in the external medium (2.6 mM), and high inside the cell (125 mM). The Cl^- concentration is 1.2 mM and 77 mM inside and outside respectively (Hodgkin, 1951). As a general rule, the interior is some 10 to 100 times richer in K^+ and about 10 times poorer in Na^+ (Grundfest, 1961).

Besides the chemical gradient there also exists an electrical gradient across the cell membrane, with the interior of the cell being negative with respect to the outside. Although these gradients occur across the cell membrane, yet the membrane does not maintain this electro-chemical gradient by simply being impermeable to the ions involved. The ions tend to leak under the influence of their electrical and concentration gradients, but the so-called active Na^+-transport process (or simply the 'Na^+-pump') located in the membrane ejects Na^+ outward against its electro-chemical gradient and in exchange takes up K^+. In so doing, the active Na^+-pump utilizes some of the metabolic energy of the cell (Ussing, 1949). Adenosine triphosphate (ATP) has been shown as a source of energy for the Na^+-pump (Caldwell, 1960).

(C) The Resting Potential:

The resting plasma membrane is sparingly permeable to sodium ions and freely permeable to potassium and chloride ions, which exchange across it solely by diffusion. Since the interior of the cell contains a large quantity of non-diffusable anions (A^-), K^+ and Cl^- are distributed very unequally under the conditions of Donnan equilibrium (Boyle and Conway, 1941; Conway, 1946, 1947). The internal potassium concentration is approximately 20 to 50 times higher than in the external fluid, while an inverse relationship holds for chloride distribution as shown by the following equation.

$$\frac{(K)_i}{(K)_o} = \frac{(Cl)_o}{(Cl)_i} = 20 \text{ to } 50 \qquad (1)$$

where $(K)_i$ and $(K)_o$ are the concentrations of potassium ions respectively inside and outside and similarly for chloride ions (Eccles, 1953). The transmembrane potential arises because the membrane is much more permeable to K^+ than it is to Na^+ and because the Na^+-K^+-pump maintains the internal Na^+ concentration at a low value. K^+ would diffuse out of the cell faster than Na^+ would diffuse into it if there were no membrane potential, K^+ diffusing out must leave the non-permeating anion (A^-) behind and thus the membrane would become electrically polarized, inside negative (Woodbury, 1965). The chemical nature of the intracellular anions has not been completely elucidated. However, Conway (1950) is of the opinion that in the muscle the negative charges are supplied largely by the organic phosphates and amino

acids. In the case of squid giant axon it is maintained that a large quantity of non-diffusable anions is provided by isethionate which is present in a concentration of 270 mM; in other nerve fibres, glutamic and aspartic acids have been reported in substantial quantities (Katz, 1966).

In nerve and skeletal muscle, the resting potential closely corresponds to the K^+ equilibrium potential, E_K, and the magnitude of the potential can be expressed by the Nernst equation (Hodgkin and Huxley, 1952),

$$E_K = \frac{RT}{ZF} \ln \frac{(K)_i}{(K)_o} \qquad (2)$$

where R is the gas constant, T is the absolute temperature, Z is the valency of the ion, and F is the Faraday's constant, 96,500 international coulombs. When Z is unity, and the temperature is 20°C, the formula becomes:

$$E_K \text{ (in mV)} = 58 \log_{10} \frac{(K)_i}{(K)_o} \qquad (3)$$

Thus for a Donnan ratio of 20 to 50 it would be predicted that the outside would be 75 to 100 mV positive to the inside. Such a potential compensates for the concentration gradient so that the electro-chemical gradient for potassium or chloride ions is virtually zero, i.e. no work is done if a small quantity of K^+ or Cl^- is transferred in either direction across the membrane, or alternately, if the concentration (or more precisely the activity) of K^+ is say fifty times greater inside

than outside, K^+ will bombard the membrane fifty times more intensely on the inside; hence a potential difference (about 100 mV) across the membrane is necessary in order to equalize the inward and outward potassium fluxes. If the potential is less than this value, there will be an excess outward flux of potassium ions each carrying a charge, until the equilibrium potential is attained, and vice versa if the potential is greater than the equilibrium value (Eccles, 1953).

Conway (1957) pointed out that the Nernst equation should hold for the "balanced state" or condition of zero net flux. When we substitute in this equation the K^+ concentrations of fibre water in freshly dissected frog sartorius muscles, $(K)_i$, and that of normal Ringer's fluid (with composition similar to that of frog plasma), the calculated K^+-equilibrium potential is about 100 mV. However, the injury potential measured in this fluid was less than 60 mV, and the difference between observed and theoretical potentials was attributed to short-circuiting around the electrodes and to the poor condition of the tissue.

(D) Testing the Applicability of the Nernst Equation:

Ling and Gerard (1949), using a micro-capillary filled by 3M KCl found a resting membrane potential of 97.6 $\pm$ 5.7 mV for frog sartorius muscle fibres immersed in normal Ringer's fluid. This recorded membrane potential agrees quite closely with the calculated potential. Equipped with the useful microelectrode technique,

the next step in determining whether the resting potential of nerve and muscle was a potassium diffusion potential was to vary some of the parameters in Nernst equation and compare the measured and calculated potentials under these conditions. For example, if the K^+ concentration gradient across the cell membrane is kept constant the observed membrane potential should, according to the equation, be proportional to the absolute temperature. This was found to be the case by Ling and Woodbury (1949) who obtained a Q_{10} of 1.033 for muscle over the temperature range of 4-30°C. With the same preparation Jenerick and Gerard (1953) found a value of 1.05, indicating also that the potential was generated by a purely physico-chemical as distinct from enzymatic process. In the latter case Q_{10} values of 3 or more might be expected.

The membrane potential of resting nerve and muscle has also been measured in relation to changes in the potassium concentrations of the cell fluid, $(K)_i$, and external bathing fluid. Consider the case in which $(K)_i$ was kept constant and $(K)_o$ was varied: when $(K)_o$ was increased in the bathing medium in such a manner as to keep the product $(K)_o \times (Cl)_o$ constant, no KCl entered the fibres and therefore $(K)_i$ did not change. The following equation would be expected to apply:

$$E_m = E_K + 58 \log_{10} \frac{1}{(K)_o} \qquad (4)$$

where E_m is the measured potential following addition of potassium of concentration $(K)_o$ to the fluid bathing the tissue, and E_K is the

K-equilibrium potential before the addition of K^+ (Kernan, 1965). So taking into consideration the above equation, if one plots the measured membrane potential against the $\log_{10}$ of $(K)_o$, a straight line with a slope of 58 mV for a tenfold change in $(K)_o$ should be obtained.

Boyle and Conway (1941), and Hodgkin and Horowicz (1959) along with others have confirmed the validity of the Nernst equation and have presented experimental evidence that in the skeletal muscle the resting membrane potential is a K-diffusion potential. Hodgkin and Horowicz (1959) kept the product $(K)_o \times (Cl)_o$ constant by substituting sucrose or sulphate for chloride in the Ringer's fluid, and they increased $(K)_o$ in substitution for $(Na)_o$. They obtained a straight line graph of E_m plotted against $(K)_o$ with a slope of 58 mV over the $(K)_o$ range of 10-100 mM. The intracellular K concentration may also be kept constant where K^+ is substituted for Na^+ in the medium in the presence of an impermeable anion. Where this was done Equation 4 was found to apply over the $(K)_o$ range of 5-100 mM (Kernan and Conway, 1955).

A serious criticism has been raised against the hypothesis that the resting potential in muscle and nerve is a potassium diffusion potential. The membrane potential does not always respond to changes in the external K^+ concentration. Although as indicated above, the Nernst equation applied very well at $(K)_o$ of 5 mM or more. At low K^+ concentrations the curve deviates widely from the predicted linear relationship. Hodgkin and Katz (1949) have shown that this deviation is to be expected because the excised tissues are not in the steady

state that would prevail *in vivo*, but are gaining Na^+ and losing K^+. Further they showed that in such a non-steady state the resting membrane potential, Er, would be given approximately by an equation derived from the constant-field theory of Goldman (1943):

$$Er = \frac{RT}{F} \ln \frac{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i} \qquad (5)$$

Where $[K]_i$, $[Na]_i$ and $[Cl]_i$ are the activities (concentration x activity coefficients) of the ions inside the cell and $[K]_o$, $[Na]_o$ and $[Cl]_o$ are the activities of ions outside the cell; and P_K, P_{Na} and P_{Cl} are the permeability constants for the respective ions. When the assumed relative values for P_K, P_{Na} and P_{Cl} are used in plotting the curves, a good agreement with the observed potentials becomes quite apparent. Thus with high external potassium concentration the relationship approximates to the predictions made by the Nernst equation, but with low potassium concentrations the fraction of the potential attributable to the sodium and chloride ions dominates the contribution by the potassium ions, and, with potassium concentrations lower than that normally obtaining *in vivo*, the observations deviate widely from prediction.

In vivo measurements of resting potential were made by Moore and Cole (1960) in the giant nerve axon of the squid. They found a value of 77 mV which was close to the K-equilibrium potential. The potential was also measured in mammalian muscle *in vivo* (Bennett *et al.*, 1954), where the value found was 99.8 $\pm$ 0.19 mV. Here surprisingly the calculated potential was only 95 mV, and the difference was

attributed to the activity of an electrogenic Na^+-pump. It is possible that here $(K)_i$ was underestimated when given a value of 140 mEq/L fibre water (Hastings and Eichelberger, 1937). Kernan (1965) points out that a more reasonable value for dog muscle would seem to be 160 mEq/L, which would give an E_K value of 97.5 mV, this being not significantly different from that measured. It may therefore be concluded that as one approaches in vivo conditions the Nernst equation holds quite well for the nerve and muscle.

It has also been shown that muscle bathing in plasma or serum instead of Ringer's fluid takes up far less Na^+ (Carey and Conway, 1954; Creese et al., 1958), and the potential measured in animal's own plasma approaches very close to that of the calculated value. For an instance, the mean resting potential of frog sartorius muscle fibres measured in plasma was 99.2 $\pm$ 0.7 mV, where the calculated potential was 100.1 mV (Kernan, 1960). The membrane potential of the extensor digitorum muscle of rat, measured in plasma by the microelectrode, with adequate oxygenation, was 90.1 $\pm$ 0.69 mV compared with a calculated value of 91.0 mV, based on K analysis of the tissue (Kernan, 1963).

While the Nernst equation seems to apply to the nerve and skeletal muscle at rest, there are other tissues, such as smooth muscle, where the equation does not seem to apply. The mear membrane potential in the smooth muscle of the taenia coli of guinea-pig, for example, measured by microelectrode was at least 20 mV lower than the calculated K diffusion potential (Bülbring, 1954), and the line obtained when E_m was plotted against $(K)_o$ had a slope of only 38 mV per tenfold

change in concentration, instead of 58 to 60 mV predicted by the ionic theory. Smooth muscle responds to mechanical pressure and stretch with depolarization and the appearance of spontaneous electrical activity. In taenia coli, for example, the membrane potential may fall from 60 to 43 mV on stretching. Skeletal muscle behaves in a similar manner when immersed for some time in a Ca^{++}-free fluid, and this appears to be due to an entrance of Na^{+} into the muscle fibres (Kernan, 1965). In this case the balanced state with respect to ion fluxes does not exist, and so we would not expect the Nernst equation to hold. The more general Goldman constant field equation may in some cases be used to describe the relationship between the membrane potential and the relative permeabilities and electrochemical gradients of the various ion species across the cell membrane. Such a relationship has already been described in Equation 5.

(E) Action Potential:

According to the ionic hypothesis of Hodgkin and Huxley (1952) the action potentials in nerve and skeletal muscle are brought about by a transient and specific increase in sodium conductance. When the membrane is stimulated (depolarized) by an applied cathodal current or by the flow of electric currents in local circuits due to a change in membrane potential in some adjacent part of the fibre, the surface membrane immediately develops a brief and a highly specific increase in the membrane's permeability to sodium ions, presumably due to the operation of a 'sodium carrier' mechanism. As a consequence sodium

ions are driven inwards both by the concentration and voltage gradients, their positive charges rapidly discharging the resting potential. Thus, eventually the potential approaches that of a sodium electrode, the inside becoming momentarily positive with respect to the outside (reversed potential or overshoot). In skeletal muscle fibres, for example, the potential changed from a resting value of about -88 mV to a value of +31 mV, that is, by 119 mV (Nastuk and Hodgkin, 1950). The transient change of membrane potential by virtue of which the interior becomes positively charged for a few msec is called the action potential. As the peak of the action potential is reached, the sodium-carrier is already getting inactivated, and soon completely fails while simultaneously the potassium permeability is increased far above the resting level. There is thus a rapid net outward flux of potassium ions, because during the falling phase of the action potential, the 'potassium carrier' operates effectively in this direction along the electro-chemical gradient for potassium and each potassium ion carrying a positive charge outwards. In this way the original resting potential is restored in a few msec. At this level there is, according to the hypothesis, a zero electro-chemical gradient for potassium.

Theoretically during the action potential the sodium entry could continue to overpower all other ion movements until the cytoplasm acquires sufficient positive potential (about +50 to +60 mV relative to the outside) to balance the chemical potential gradient of Na ions. In practice, this level is never quite attained during the course of an action potential because (1) the opening of Na^+ channels is only a

brief transient event and (2) it is rapidly followed by an increase in the K^+ conductance, which begins to operate near the peak of the action potential and accelerates the return of the system to its resting condition (Hodgkin, 1958).

(F) Propagated Action Potential:

On the basis of membrane theory (Hodgkin, 1964), action potential is explained by a cycle of permeability changes at any region of the membrane. As the impulse advances, the potential difference across the membrane just ahead of the active region is depolarized by electric currents flowing in a local circuit through the axoplasm and external fluid; this causes a rise in Na conductance, Na^+ enters the fibre making the inside positive and giving the current required to activate the next section of the fibre. This sequence of events moves regularly along an unmyelinated axon or muscle fibre to its end. Thus, the self-propagating nature of the nerve impulse is due to circular current flow and successive electrotonic depolarizations to the firing level of the membrane ahead of the action potential.

It may be pointed out that in the myelinated nerves the action potentials are generated only at the nodes. When a fibre is depolarized only the nodes become active, hence the conduction is saltatory in myelinated nerve fibres (Huxley and Stämpfli, 1949).

The impulse moves along the nerve or muscle fibres in a non-decremental fashion, that is, at a constant amplitude and velocity of 1-100 meters per second, the speed depending on the fibre diameter,

temperature and on whether or not the fibre is myelinated (Hodgkin, 1964). The action potential must then be supplied by energy while in motion, and it seems that the immediate source of energy for propagation of the impulse is the breakdown of the electro-chemical ionic gradients which exist across the cell membrane. Therefore, in the active state of the fibre there is an uptake of a small quantity of sodium ions and the loss of a small quantity of potassium ions. Ultimately there must exist a mechanism which actively transports Na^+ out of the cell against its electrical and chemical gradients; the potassium ions are probably passively absorbed. Metabolic energy is thus eventually required for a complete restoration of the status quo, the sodium ions gained in bursts of activity being slowly pumped out later during periods of rest.

(G) Effect of External Sodium Concentration on the Action Potential:

One of the crucial tests of the sodium hypothesis was to vary the sodium concentration in the bathing medium and to study the effect of sodium ions on the amplitude, on the maximum rate of rise (dv/dt) and on the overshoot (reversal potential) of the action potential. Since the magnitude of all these parameters depends upon the sodium conductance across the plasma membrane a series of investigations have been made in which the effect of sodium ions have been studied on different types of preparations. For example, on squid giant axon (Hodgkin and Katz, 1949), on frog medullated nerve fibres (Huxley and Stämpfli, 1951), on the frog skeletal muscle (Nastuk and

Hodgkin, 1950; Desmedt, 1953), on conduction bundle of the dog heart (Draper and Weidmann, 1951), on the frog ventricle (Brady and Woodbury, 1960), and on the skeletal muscle of the guinea pig (Ferroni and Blanchi, 1965). On all these preparations the importance of external Na^+ concentration in the generation of the action potential spike has been shown. The following discussion will mostly be confined to the effect of sodium ions on the squid giant axon and the skeletal muscle fibres.

While studying the effect of sodium ions on the electrical activity of squid giant axon, Hodgkin and Katz (1949) demonstrated that: (1) replacement of external Na^+ by sucrose or choline reversibly reduced the size of the action potential and abolished excitation; (2) height of the overshoot of the action potential varied linearly with the logarithm of the external Na^+ concentration; and (3) a reduction in Na^+ concentration caused a reversible decrease in the rate of rise of the action potential. Subsequently it was shown by Hodgkin and Keynes (1956) that the height of the action potential was also decreased, when internal Na^+ concentration in the squid axons was raised by microinjection. From these observations it seemed likely that the membrane potential at the crest of the spike was determined by Na^+ rather than by K^+ concentration gradient across the cell membrane. This indicated that during the rising phase of the action potential the membrane became more permeable to Na^+ than to K^+ and Cl^-, and that the membrane potential approached the Na-equilibrium potential defined by:

$$(E_a)\ \text{test} - (E_a)\ \text{Ringer's} = 58\ \text{mV.}\ \log_{10}\frac{[Na^+]\ \text{Ringer's}}{[Na^+]\ \text{test}} \qquad (6)$$

where (E_a) test and (E_a) Ringer's are the potential differences across the active membrane in the test solution and in the normal solution (Ringer's fluid or sea water). $[Na^+]$ test and $[Na^+]$ Ringer's are the sodium concentrations in these solutions. This equation would be obeyed if the active membrane behaved like a sodium electrode and the internal sodium concentration did not change appreciably during the period of the experiment (Hodgkin, 1951). Where the internal charge is positive, both the concentration and electrical gradients favour the net efflux of K^+ from the cell, which would tend to repolarize the membrane.

Nastuk and Hodgkin (1950), and Desmedt (1953) have confirmed the validity of the sodium hypothesis on the sartorius muscle of the frog. Their results indicate that the magnitude of the action potential and overshoot of the spike are also linearly proportional to the logarithm of the sodium concentration in the external fluid. The experimental points fit reasonably well on the straight line drawn according to the Nernst formula: $58 \log_{10}[Na^+]_i/[Na^+]_o$. Deviations from linearity at low internal concentration are to be expected because the sodium permeability at the peak of the action potential is not likely to be infinite with respect to the permeability to other ions (say K^+ or Cl^-) whose contribution would tend to reduce the actual overshoot. This effect would indeed be more appreciable when the ratio $[Na^+]_i/[Na^+]_o$ becomes very small (Hodgkin and Katz, 1949).

Hodgkin and Katz (1949) predicted that the rate of rise of the action potential should be determined by the rate of entrance of sodium ions into the membrane, and to a rough approximation the rate of rise should be directly proportional to the external concentration of Na^+. Their experiments with squid giant axon showed that the rate of rise of the action potential undergoes a large and substantially reversible change as a result of treatment with sodium-deficient solutions. On the other hand, substantial increases in the rates of rise were noticed in solutions containing extra sodium. The average value for the maximum rate of rise of the spike found was 630 V/sec. The experimental findings of Nastuk and Hodgkin (1950) and Desmedt (1953) with the frog muscles showed that the rate of rise of the action potential is also likewise influenced by the Na^+ concentration in the bathing medium. The maximum rate of rise in the frog skeletal muscle in normal Ringer's solution (containing 104 mM - Na) is of the order of 300 to 400 V/sec (Desmedt, 1953). The general result is therefore clearly consistent with the idea that the active depolarization of a nerve or muscle fibre is due to entry of sodium.

(h) Subthreshold Activity and the Threshold for Excitation:

There is now a general agreement that a cathodal stimulus too weak to generate a propagated impulse will produce a local or graded response that resembles a miniature action potential (Katz, 1937; Hodgkin, 1938; and Hodgkin et al., 1949). As Hodgkin (1951) points out, the subthreshold response may be simply explained, if one

considers that there exists a graded and reversible relation between membrane potential and sodium permeability. With a small depolarization, sodium permeability (P_{Na}) rises only to a small extent, so that the inward current due to sodium entry is less than the outward currents due to the movements of the potassium and chloride ions. Under these conditions the membrane activity will die out and the membrane would repolarize. On the other hand, if the applied depolarizing current is made slightly larger so that threshold of the fibre has been reached, the net Na^+ influx through the membrane slightly exceeds the sum of the net K^+ efflux and the net Cl^- influx, resulting in a net movement of positive charges into the fibre and the action potential upstroke ensues in a cyclic fashion: A decrease in membrane voltage acts to increase P_{Na} and this increase, in turn reinforces additional depolarization.

According to this view, the membrane threshold is defined by the potential of depolarization at which the inward sodium current exceeds the outward potassium and chloride current and so initiates a self-regenerative action. This critical voltage for excitation is about 15 mV in the squid axon, and a graded response is expected below this level of depolarization (Hodgkin et al., 1949). Once the threshold is reached, the fibre will fire an action potential which will reach a maximal value. Any further increase of a depolarizing current (supra-maximal stimulus) will not increase the amplitude of the spike. Thus the all-or-nothing nature of the propagated action potential immediately follows from the sodium mechanism.

(I) Limitations of the Sodium Hypothesis:

Although the action potential of excitable tissues is invariably accompanied by an increase in the conductivity of the membrane, it does not always depend on an inward movement of sodium. It was shown by Lorente de Nó (1949) that certain quarternary ammonium ions can replace sodium and, as was originally demonstrated by Overton (1902), e.g. lithium is always an effective substitute. These experiments showed that cells which normally function with sodium can use some substitutes, but there are tissues in which deviations from the typical sodium electrode type behaviour have been observed. For example, crab muscle fibres previously treated with tetraethylammonium ions not only maintain the spike but give large overshoot in the absence of sodium or any other monovalent cation, so that the Na-theory clearly does not apply in this case (Fatt and Katz, 1953). Bülbring and Kuriyama (1963) are of the view that in smooth muscle, under appropriate conditions, calcium ions may provide the inward current.

Exceptions to the application of Na-theory *in toto* have also been found in guinea-pig ventricles treated with solution in which Na^+ was replaced by choline (Coraboeuf and Otsuka, 1956), and in toad sartorius muscle immersed in Ringer's containing tetraethylammonium ions (Hagiwara and Watanabe, 1955). In both cases fibres failed to conduct action potentials in the complete absence of Na^+, but at intermediate concentrations the changes noticed in overshoot were much less than would be expected from the sodium theory. Nevertheless, in spite of these exceptions, the sodium-potassium mechanism is applicable in

majority of excitable tissues, and the effects of Na^+-deficient solutions agree well with the hypothesis that the action potential depends on an increase in sodium permeability.

CHAPTER II

THE EFFECTS OF ANESTHETIC DRUGS ON THE PERMEABILITY CHARACTERISTICS OF EXCITABLE MEMBRANES

CHAPTER II

THE EFFECTS OF ANESTHETIC DRUGS ON THE PERMEABILITY CHARACTERISTICS OF EXCITABLE MEMBRANES

(A) General Comment:

There is hardly any area of pharmacology in which as many theories for the mode of drug action have been advanced as in the field of anesthetics. Extensive research efforts have been devoted towards this problem, and a number of physico-chemical and biochemical hypotheses proposed to explain the mechanism of conduction block by local and general anesthetics. Several reviews (Verworn, 1913; Lillie, 1923; Höber, 1945; Pittinger and Keasling, 1959; Watson, 1960; Pauling, 1961; Quastel, 1962; Bunker and Vandam, 1965) provide voluminous literature pertaining to the various theories of anesthesia. In view of the present investigation, attention in this introduction will be focused mainly on the salient features of the permeability theory and the main criticism that has been raised against the permeability theory without entering into a detailed consideration of other theories of narcosis .

(B) Cell Permeability Theory of Anesthesia:

One of the principal proponents of the 'Permeability Theory of Narcosis' was Lillie (1923). Most of his work was done on the eggs of the sea urchin Arbacia and the pigment-containing larvae of Arenicola. Lillie found that the permeability of the Arbacia eggs to

water was decreased when the narcotics were added to the diluted sea water. His observations with the Arenicola larvae showed that these organisms ordinarily lost their pigment to the surrounding medium when transferred from sea water into isotonic saline. Such a loss of pigment was thought to be due to a marked increase in cellular permeability. Correspondingly, no immediate loss of pigment was noticed from larvae immersed in solutions containing suitable concentrations (in the anesthetizing range) of ether, chloretone and alcohol. More direct studies of the effect of narcotics on permeability were done earlier by Winterstein (1915), who measured the rate of increase of weight of frog sartorius muscles in hypotonic salt solutions. He found that the rate of increase, i.e. the permeability to water, was lower when ethyl alcohol was added to the soaking medium. At about the same time Osterhout (1913) demonstrated that the electrical resistance of marine plant cells was reversibly increased in the presence of anesthetics like chloroform, ether, chloral hydrate and ethyl alcohol and such an effect was again attributed to a decrease in the permeability of the cell membrane to ions.

Impressed by his own observations, and from the contemporary supportive evidence of other investigators, Lillie postulated that in the presence of narcotizing drugs the cell membrane was modified in such a manner that stimulation could not produce the normal rapid increase in permeability required for the ionic exchanges occurring with depolarization. A further conclusion drawn from these studies was that the narcotic agents produced central nervous system (CNS)

depression because they interfered with the movement of ions so necessary for the transmission of the nervous impulse.

As with all other theories of narcosis, criticism has been raised against Lillie's theory and some inadequacies and exceptions reported (Brooks, 1947; Davson and Danielli, 1952). There has been much doubt as to the effect of narcotics on permeability, and it appears that there is no general rule. In a preliminary communication of their findings, Jacobs and Parpart (1937) indicated that n-butyl alcohol (15.6 to 250 mM) markedly decreased the permeability of glycerol in the erythrocytes of man, rabbit and rat, etc.; while in the erythrocytes of ox, horse, pig, dog and cat, etc., the permeability to glycerol was increased by n-butyl alcohol. Bärlund (1938) showed that ethyl ether (1 to 2.5 vols % solns) reversibly increased the permeability of the Chara cells (brackish alga) to ethylene glycol, urea etc., and decreased the rate of intake of lithium ions. Similarly, Liebe (1948) showed that phenyl-urethane induced a reversible decrease of permeability of cow and horse erythrocytes, however, this narcotic did not influence the permeability of human red cells. In another report, Haglund and Lovtrup (1966) indicate that butyl alcohol enhances the permeation of radioactive water into amphibian eggs, whereas procaine and nupercaine depress the permeability. Some of these exceptions to the permeability theory seem to be valid, where sophisticated techniques for determining the permeability coefficients were employed, and the concentrations of the anesthetics used were within the pharmacological limits. In other instances the increase in perme-

ability by the anesthetic agents may be due to: (a) species difference, because the erythrocytes of some species show a narcotic effect with regard to certain solutes and not with regard to others; (b) higher concentrations of the narcotics used which would damage the cell membrane and thus may change its permeability characteristics; (c) in some other cases the compositions of the control and drug containing media were not strictly physiological and this might account for the observed discrepancies. In general, the evidence in the literature indicates that most of the narcotic drugs inhibit permeability of plant and animal cells including artificial membranes to electrolytes and nonelectrolytes. A few of the objections raised against Lillie's theory of narcosis seem to be untenable, since the work that tends to disprove the general validity of this theory was based on experiments carried out to demonstrate that narcotics did not always influence or decrease the cell permeability in all cells, and it is not applicable to Lillie's essential concept. The latter being essentially concerned with the very special changes in permeability that accompany excitation in the tissue. Moreover, Lillie advocated that a narcotic would prevent the conduction of an impulse along a nerve, not because it altered the normal permeability relations in the resting nerve, but because it prevented the supposed increase in permeability which is associated with the passage of the impulse. It is, however, worthy of note that compounds which enhance membrane permeability *in vitro* (e.g. n-butyl alcohol and ethyl ether), and produce CNS depression when injected into the body show that the mechanism of action cannot be the

same for all substances exerting effects of this kind. In concluding, it may be stated that as far as the local and general anesthetics are concerned, the fundamental question which has been posed time and again by the permeability theory is whether or not an alteration in cellular permeability which occurs as a result of the application of anesthetic drugs on the neural and non-neural membranes is responsible for the decreased excitability or the conduction block of the system being studied. Before presenting some of the electrophysiological studies which support this hypothesis, some of the indirect evidence which has emerged from the studies of monolayer model systems is considered in the succeeding section.

(C) Monolayer Model Systems and the Anesthetic Action: In 1945, Rideal pointed out that the monolayer (monomolecular layer) model may be the best physical representation of the membrane for studying anesthetic action. The need for such a model system arose from a poor comprehension of the highly organized and complex nature of the plasma membrane and the obvious simplicity of model membranes. The model membranes are, of course, in no way the true representatives of living membranes, but they have helped in the study of the effects of ions and drugs at physiological and pharmacological concentrations. Responses and antagonisms are produced which are sufficiently distinctive to suggest interactions related to biological effects.

Shanes (1962) suggested that the permeability changes induced by pharmacological and physiological agents and the various antagonisms

that have been demonstrated represent criteria that must be satisfied by any model or hypothesis proposed to account for the nature and the properties of excitable membranes. An important clue to a suitable model was provided by Skou (1958, 1961), and in a series of investigations, Skou demonstrated that the potency of a wide variety of agents that block nerve conduction closely parallels their ability to interact with monomolecular films of stearic acid and especially with films made of myelin extracts. This interaction, he believed, was caused by adding the bulk of the anesthetic agents to the films, thereby increasing molecular packing and hence the surface pressure (force) as measured with a Langmuir film balance. In a subsequent study, Skou found that a group of excitatory agents (e.g., veratrum alkaloids) decreased the spreading force in a lipid monolayer of nerve tissue. The increase in the spreading force produced by the local anesthetics, and therefore the increase in the lateral pressure in the membrane may lead, as Skou visualized, to a decrease in the effective size of the pores for sodium movements in the excited membrane and hence to a decrease in the permeability for sodium ions. For the drugs which reduce surface pressure, the reverse situation would be assumed.

The significance of the stearic acid monolayer membranes has been appreciably strengthened by the findings of Gershfeld and Shanes (1959), and Shanes and Gershfeld (1960). The conclusions drawn by these workers regarding the permeability changes in the membrane to Na^+ and K^+ by the local anesthetics (stabilizers) and veratrine and related alkaloids (labilizers) are almost identical to those of Skou

(1961). Toman (1962) has described another model of the neural membrane, in which a sheet formed of hydrogen-bonded polypeptide chains is held in folds by linkages between acid residues. The critical event in excitation is assumed to be the limited diffusion of sodium ions into the folds. According to this view, the action of local anesthetics and related drugs which cause an elevation of neural threshold is accompanied by forming multiple stable linkages between the amino acid residues and the drug (stabilizing action), which somehow decreases sodium influx. There is at present no direct proof for the details of any of the above mechanisms. Whatever interactions between the drug and membrane occur, they seem to do so in such a way as to present a barrier to sodium influx, and to make the active membrane more resistant to the movement of Na^+.

Since the phospholipids form an important structural component of the cell membrane, the interaction or binding of local anesthetics (Feinstein, 1964; Feinstein and Paimre, 1966), the general volatile and gaseous anesthetics (Clements and Wilson, 1962, 1963), and the neurotropic agents such as chlorpromazine and diphenhydramine (Ehrenpreis, 1964) has been studied with the synthetic phospholipids (cephalin and lecithin) and the phospholipids extracted from the cellular membranes. The results of Feinstein (1964) indicated that local anesthetics such as procaine, tetracaine and butacaine react with the phosphate group of phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl inositide, lecithin and cephalin. The binding ratio between the local anesthetic and phospholipid was found to be 1:2, i.e. com-

plexes composed of one mole of local anesthetic and two moles of phospholipid were formed. His experiments also showed that the local anesthetics increased the electrical resistance of the model membranes prepared by impregnating the millipore filters with cephalin plus cholesterol or tissue lipids extracted from the rabbit skeletal muscle microsomes. On the basis of these observations Feinstein (1964) suggests that the interaction of the local anesthetics with the phosphate groups of the cell membrane phospholipids may be responsible for impeding the cellular ion fluxes, and such an interaction may provide a chemical basis for the ability of the anesthetic drugs to inhibit nerve conduction.

Hille (1966) is of the view that the sodium channels of the nerve are closed or clogged by the binding of the anesthetic molecules to a complementary structure in the channel. He further points out that probably the sodium channel bears a negative charge that attracts sodium ions, calcium ions and other cationic molecules including the anesthetics. The active form of the anesthetic molecule is believed to be a cation and approaches the binding site from the extracellular side of the cell membrane. According to this suggestion, the anesthetics decrease the magnitude of the sodium current because the anesthetic molecules interact or bind with the sodium channels and thus in turn decrease the available number of channels for the sodium ions.

(D) The Effects of Anesthetics on the Electrical Properties of Excitable Cells:

As described above, according to the ionic hypothesis which Hodgkin and Katz (1949) have formulated, the sodium ions play a key role in the initiation of an electrical impulse. It is, therefore, not unreasonable to assume that any theory of anesthesia attempting to explain a unified mechanism of action of anesthetic drugs must take into consideration the possible depression of Na^+ influx as the cause of conduction block of impulses. Evidence has been accumulating in recent years in favour of this type of hypothesis. It has been found that drugs which produce local and general anesthesia, affect the bioelectrical signals by decreasing the increase in sodium conductivity of the cell membrane following a stimulus (Thesleff, 1956; Taylor, 1959; Yamaguchi, 1961; Inoue and Frank, 1962, 1965, 1967; Frank and Sanders, 1963; Yamaguchi and Okumura, 1963; Frank and Pinsky, 1966; Inoue et al., 1967).

The development of intracellular recording techniques with microcapillary electrodes, of voltage-clamp techniques and of sucrose-gap methods have provided a further impetus for the re-appraisal of the action of local and general anesthetics. Inoue and Frank (1962, 1965) studied the mechanism of action of procaine and ether on the isolated sartorius muscles of the frog using the extracellular and intracellular recording techniques. Their results with extracellular electrodes showed that in procaine and ether-treated muscles the maximum amplitudes of the compound action potentials were decreased

and the thresholds for stimulation were increased as compared with the controls. Measurements obtained with intracellular microelectrodes also revealed the changes expected from a reduction in the specific increase in Na^+-conductance upon stimulation. For example in the drug treated muscles it was observed that: (i) the maximum rate of rise of the action potential was decreased; (ii) the overshoot of the spike was reduced or abolished; (iii) the threshold for membrane depolarization was increased; and (iv) the transverse membrane resistance was increased with ether, but remained unchanged with procaine. Only small changes in the resting membrane potential were recorded at blocking concentrations of the drugs.

Since sodium is known to have an important function in the genesis of the action potential, and since the maximum rate of rise and overshoot of the action potential are roughly dependent upon the logarithm of the sodium concentration in the bathing medium (Hodgkin and Katz, 1949; Nastuk and Hodgkin, 1950), any agent which impedes the function of this cation will inevitably tend to interfere with spike generation and would likewise affect the other properties of an action potential. Inoue and Frank (1962, 1965) concluded that procaine and ether block excitability by antagonizing the specific increase in the sodium conductance in the fibre membrane. When they raised the Na^+-concentration in the bathing fluid to 171 mM (1.5 x normal Ringer's), they found that the overshoot of the spike and the maximum rate of rise of the action potential were restored to the control level. These findings led them to propose that local and general anesthetics have

a fundamentally similar mechanism of action; i.e., they act by inhibiting the specific increase in sodium conductivity following a stimulus. The investigations of Crescitelli (1952) and Condouris (1961) on bullfrog nerve fibres treated with certain nerve blocking drugs have revealed a similar type of interaction (competition) between drug molecules and sodium ions. The same mechanism of action was found to be operating in the skeletal muscle for pentobarbital, tribromethanol, paraldehyde, chloralose, chloral hydrate and urethane (Thesleff, 1956), ether, chloroform and urethane (Yamaguchi, 1961; Yamaguchi and Okumura, 1963).

Results reported by Weidmann (1955) with intracellular microelectrodes indicate that in Purkinje fibres procaine amide, cocaine, quinidine sulphate, and diphenhydramine stop spontaneous activity within a few minutes without appreciably affecting the membrane potential. He also found that the application of these drugs considerably decreases the rate of rise and overshoot of the action potential. Weidmann interpreted his results on the basis that the local anesthetics block conduction by interfering with the system responsible for carrying sodium ions through the membrane ("sodium carrying system"), for the effect of these drugs can be counteracted by injecting anodal currents into the fibres (increasing the membrane potential) and thereby activating the "sodium carrying system". Inoue and Frank (1965) were also able to reverse the electrical inexcitability produced by procaine or by ether by passing hyperpolarizing currents through the membrane. The procaine block, however, was

more resistant to reversal than was the ether block and thus the hyperpolarizing currents had to flow for several seconds before the procaine block could be reversed. Voltage-clamp studies on squid giant axons carried out by Taylor (1959) also have shown that procaine retards the sodium current across the membrane and the blocking action of procaine is therefore the result of an inhibition of the "sodium carrying system." Simultaneously Shanes et al. (1959) using the same technique, provided evidence supporting this mechanism of action for procaine block in squid giant axon.

Straub (1956a, 1956b) studied the effect of procaine on the myelinated nerve fibres of frog using the sucrose-gap method, and came to the conclusion that the main effect of local anesthetics seems to be due to the decrease of sodium permeability. Schoepfle and Grant's (1954) conclusion regarding the effect of novocaine on amphibian nerve is also consistent with this view. The evidence presented thus far strongly supports the hypothesis that the prime action of anesthetic drugs is to reduce the specific increase of Na^+-conductance in the membrane, the resting membrane potential remaining practically unchanged at blocking concentrations of the anesthetics.

(E) Statement of the Problem:

The drugs used in the present investigation are classified according to their current therapeutic use as tranquilizers, analgesics, anticholinergics, antihistaminics, sedatives and hypnotics. When used in therapeutic doses, these drugs produce a nonspecific depression of

the central nervous system (CNS), and some like the antihistamines are also capable of producing local anaesthesia (Ritchie et al., 1965), for which purpose they have been used occasionally. The nonspecific central depressant effect of these drugs seems to be due to an inherent anesthetic property of these neurotropic compounds (Jhamandas, 1969). Nevertheless, no clearcut explanation for the mechanism of central depression by all these drugs exists, and likewise, the mechanism for local anesthetic effect produced by some of these compounds is not known.

The aim of the present investigation was to study the effects of a wide variety of chemically unrelated neurotropic compounds, capable of producing CNS depression, on excitability in skeletal muscle fibres. An important objective was to see if their mechanism of action can also be explained on the basis of sodium-suppressant mechanism as has been proposed by Inoue and Frank (1962, 1965). According to this hypothesis, the local and general anesthetics possess a common mechanism of action and depress excitability in the neural and skeletal muscle membranes by decreasing the specific increase in sodium conductance responsible for the generation and propagation of the action potential. Experiments were carried out to study the effects of drugs on the isolated sartorius muscles of the frog using extracellular electrodes and intracellular microelectrode techniques.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III. MATERIALS AND METHODS

(A) Muscle Preparations:

The sartorius muscles of the frog, Rana pipiens, were used throughout the present investigation. Each frog was decapitated, pithed and the sartorius muscle was carefully dissected and removed from the frog with a portion of the pelvic girdle containing the proximal attachment of the muscle. After its removal the muscle was placed in a dissecting dish and, with the aid of a dissection microscope, was freed from connective tissue and fascial membranes. The isolated sartorius muscle was mounted horizontally in Ringer's fluid with its deep surface uppermost. This was important, since the superficial surface is covered with a layer of connective tissue which hinders the insertion of microelectrodes (Nastuk and Hodgkin, 1950). In order to minimize the damage of the membrane by the microelectrode when the muscle moved, the muscle was stretched tightly in the bath. All experiments were done at room temperature (18° to 20°C).

For the experiments using extracellular recording techniques, the isolated muscle was reduced in size by successive removal of muscle fibres from both sides of the muscle until a bundle of fibres from the central portion approximately 1 mm in diameter remained. The dissection was performed with the aid of a dissection microscope, care being taken to prevent or minimize damage to muscle

fibres remaining in the bundle.

Some of the frog muscles were found infected with larvae of nematodes and trematodes. The heavily infected muscles were discarded, but the muscles in which the incidence of infection was relatively low were utilized, all the visible parasites and the proliferated connective tissue layer around them were removed. Frank (1957) observed that if the infected muscles are kept immersed in Ringer's solution for about one and a half hours the resting and active membrane potentials reach the normal values observed in uninfected frogs. Therefore, the infected muscles after mounting into the bath were soaked in Ringer's solution for 90 to 120 minutes before the actual observations were started. In contrast, the noninfected muscles were allowed to equilibrate in the Ringer's solution for about 60 minutes before the actual measurements were made.

The frogs were maintained at room temperature. Experiments were performed throughout the year, however seasonal changes did not seem to affect the results, although quantitative comparisons were not attempted.

(B) Solutions:

The Ringer's solution was prepared in distilled water which had passed through a filter to remove organic material (Barnstead 0812, Barnstead Still and Sterilizer Co.) and a deionizer (Illco-Way, Research Model Ion Exchanger, Illinois Water Treatment Co.). The composition of the Ringer's solution was as follows (mM):

NaCl, 111.8; KCl, 2.47; $CaCl_2$, 1.08; NaH_2PO_4, 0.087; $NaHCO_3$, 2.38; and glucose, 11.1. This solution had a pH of 7.4 to 7.6. Other solutions were made up with excess or reduced NaCl, the latter by substituting an equivalent amount of sucrose to maintain osmolarity. All the solutions contained d-tubocurarine (Nutritional Biochemicals Corp., Cleveland, Ohio) in a concentration of 10^{-4} g/ml to preclude possible neuromuscular effects.

For those experiments employing sodium salt of gamma-hydroxybutyric acid (GHBA), the Ringer's solutions were prepared with low NaCl so that after the addition of gamma-hydroxybutyrate the sodium content was approximately equal to that in the normal Ringer's solution (i.e. about 114 mM). Similarly, adjustments for sodium were also made when Ringer's solutions with excess or reduced sodium were prepared. The adjustment of sodium in the Ringer's solution was necessary because of the following reasons: (1) addition of sodium salt of GHBA would increase the Na^+ concentration in the Ringer's solution and thus would tend to mask the anesthetic effect produced by gamma-hydroxybutyrate; (2) further addition of sodium contained in sodium salt of GHBA would make the Ringer's solution increasingly hypertonic and thus can affect the parameters under investigation.

The osmolarities of the fluids were determined from the depression of the freezing point measured with an osmometer (Advanced Instruments Inc., Massachusetts).

(C) Drugs Used:

The drugs used in this study were:

1. Diphenhydramine hydrochloride (Sigma Chemical Co., St. Louis, Missouri).
2. Promethazine hydrochloride (Poulenc Ltd., Montreal).
3. Chlorpromazine hydrochloride (Poulenc Ltd., Montreal).
4. Hyoscine hydrobromide (Penick Canada Ltd., Toronto).
5. Morphine sulphate (British Drug Houses Canada Ltd., Toronto).
6. Meperidine hydrochloride (Winthrop Laboratories, Aurora, Ontario).
7. Gamma-hydroxybutyrate - Sodium salt - (Sigma Chemical Co., St. Louis, Missouri).
8. Gamma-aminobutyric acid (British Drug Houses Ltd., Poole, England).
9. Gamma-butyrolactone (Aldrich Chemical Co. Inc., Milwaukee, Wisconsin).

The desired concentrations of drugs were made with Ringer's solution either by mixing appropriate amounts of freshly prepared stock solutions of drugs in Ringer's fluid or by adding drugs in solid form just before use. All concentrations refer to the final bath concentration and are expressed as g/ml or in milliosmols/liter).

(D) Stimulating and Recording Procedures:

Since in this study three different stimulating and recording techniques were employed, a description of each technique along with the arrangement of the equipment used is described below. The experimental technique was essentially the same as used by Inoue and Frank (1962).

1. Extracellular Recording. The muscle bundle approximately 1 mm in diameter and about 30 to 35 mm long, prepared from the sartorius muscle was mounted horizontally on bipolar platinum stimulating and bipolar recording electrodes in a plexiglas bath. These pairs of electrodes were separated by about 22 mm and a ground electrode touched the muscle in between, the arrangement of the circuit is shown in Figure 1. Unless otherwise indicated, the muscle bundle was soaked for 30 minutes alternately in frog Ringer's solution and in Ringer's containing various concentrations of drugs. After each soaking period the bath fluid was removed with suction and the interelectrode space was dried lightly with tissue paper in order to reduce short circuiting. The muscle was stimulated with 2 msec square-wave pulses delivered through a constant current pulse amplifier (Argonaut Associates Inc., Beaverton, Oregon). At first, the strength of stimulation was gradually increased to determine the smallest stimulus required to produce a measurable action potential. This was considered to be the threshold or excitation stimulus. After such a manoeuver, 3 to 4 responses to supramaximal stimuli were recorded and finally the threshold was det-

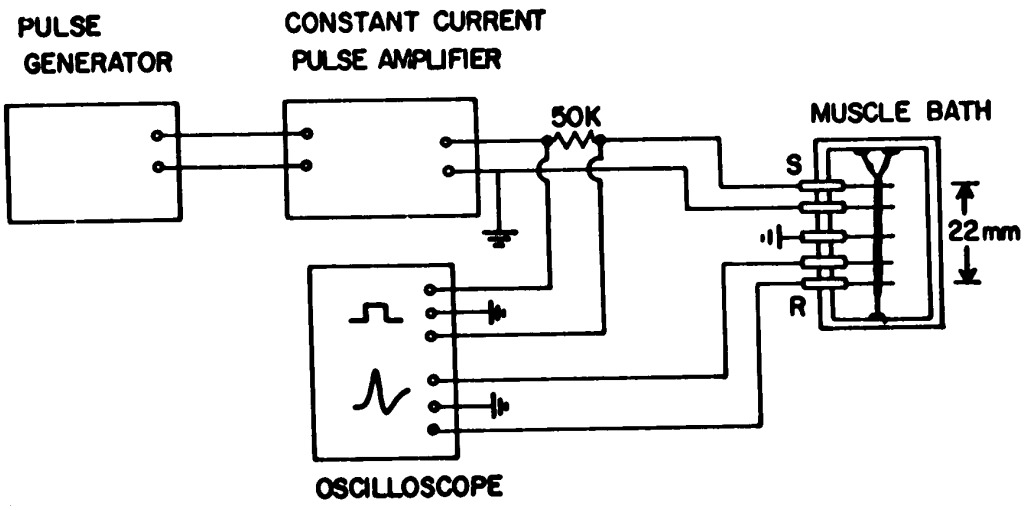

Figure 1. Block diagram of electrode assembly and muscle chamber used for testing the isolated strips of the frog's sartorius muscle. S, stimulating electrodes; R, recording electrodes.

ermined once again and the muscle placed in the next solution. These tests took between 4 to 6 minutes. The stimulating current was monitored by inserting a 50 KΩ resistance into the output of the constant current pulse amplifier and measuring the voltage across this resistance with an oscilloscope. The thresholds and action potentials were recorded with a dual beam slave oscilloscope on a 35 mm film for future measurements. The film was enlarged, and the magnified images were measured. The excitability was calculated as the inverse of the threshold current and expressed as the per cent of the control response.

ii. Extracellular Stimulation and Intracellular Recording.

For the experiments involving the measurement of the maximum rate of rise of the action potential, the whole muscle was mounted horizontally in Ringer's solution in a 20 ml plexiglas bath. This was illuminated from below and viewed from above with a binocular microscope of magnification X 50. The layout of the circuit is shown in Figure 2.

Groups of fibres were stimulated by an extracellular glass capillary electrode (pore-electrode) filled with agar Ringer's and connected through a chlorided silver wire to the stimulator. The tip diameter of such an electrode was of the order of 50 to 100 μ. When the electrode was brought in contact with the surface of the muscle with the help of a micromanipulator, the fibres being stimulated could be identified by their movement in response to stimuli. The recording microelectrode was then lowered into one of these fibres and the resting membrane potential recorded. Membrane potentials were measured

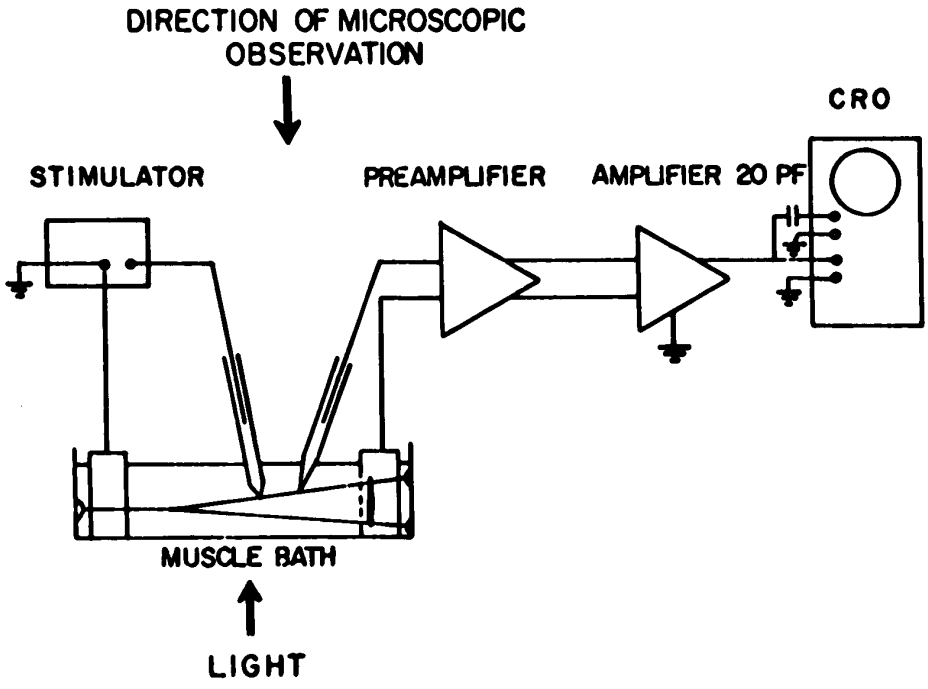

Figure 2. Diagrammatic illustration of the muscle bath and the electrophysiologic apparatus used for extracellular stimulation and intracellular recording.

to the nearest mV and, unless otherwise stated, fibres showing a resting potential less than 80 mV were not used for recording the action potential and the maximum rate of rise of the action potential. In order to minimize the stimulus artifact, the stimulating electrode was always kept at least 10 mm apart from the recording electrode. 2 msec square-wave pulses were used throughout for stimulation of the fibres.

The recording electrode had external tip diameter less than 0.5 μ and was filled with 3 M KCl (see p. 46 under Electrodes). Microelectrodes having resistance of about 10 to 15 MΩ were used. The recording electrode was connected through a silver-silver chloride electrode to the input of the cathode-follower possessing a negative capacitance (Argonaut Associates Inc., Beaverton, Oregon).

The maximum rate of rise of the action potential was determined by electrical differentiation. This was achieved by introducing capacitance (20 picofarads) into the output of the D.C. amplifier. This differentiating circuit had a time constant of approximately 20 μsec and provided an output voltage proportional to the rate of change of the input (Hodgkin and Katz, 1949). Calibration was achieved by feeding a sawtooth-wave into the input of the differentiating circuit. A standard calibration curve used for the calculation of the maximum rate of rise of the action potential is shown in Figure 3.

As in experiments with external electrodes, the muscle was exposed to the test solution for 30 minutes (unless otherwise indicated) before stimulation commenced. The bath fluid was not removed

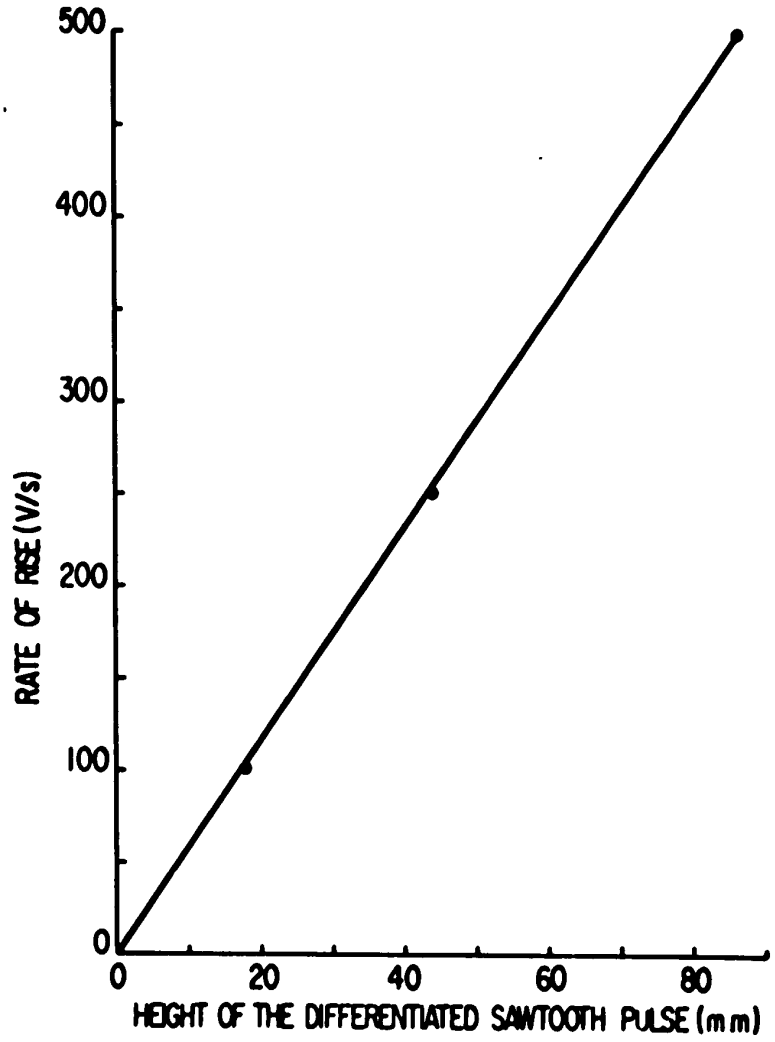

Figure 3. A standard calibration curve used for calculating the maximum rate of rise of the action potential. For description see text.

during the testing, which took about 15 minutes. Several fibres were impaled at random; the normal and differentiated action potentials were recorded on a film for analysis.

iii. Use of Two Intracellular Microelectrodes. In these final series of experiments, two intracellular microelectrodes were used. One of these microelectrodes was used to inject a stimulating current through the membrane and the other to record a potential change across the membrane. Whole sartorius muscle was used in these experiments, the preparation of which has already been described. The muscle was mounted in Ringer's solution in a 20 ml plexiglas bath in a usual way. The bath was illuminated from below and was observed from above with a binocular microscope. The outlines of the muscle fibres and the microelectrodes were best seen when two polaroid filters were used, one placed in front of the light source and the other between the objective and the muscle.

(a) Electrodes: The glass microelectrodes were drawn by a micro-pipette puller (Industrial Science Associates Inc., Ridgewood, N.Y.) using melting point capillaries having 2 mm outside diameter (Fisher Scientific Co.). The tips were examined with the high power magnification of an ordinary microscope with a dry objective. Only those electrodes having tips which could not be focused were selected for use. The selected electrodes were filled using a modified form of the method described by Tasaki et al. (1954). Before filling, the microelectrodes were mounted on a microscope slide with a rubber band

around them and were put into a jar with their tips downward. The jar was then filled with methyl alcohol and placed in a vacuum dessicator. They were vigorously boiled for 15 minutes by reducing the pressure in the dessicator. In such a manner, all the electrodes were completely filled with methyl alcohol. The jar was then filled with distilled water and the electrodes were left there overnight. Next morning they were placed in 3 M KCl and left there until used. The stock solution of 3 M KCl was filtered through a millipore filter (0.22 μ in size) before use.

Occasionally, a rapid method for filling the microelectrodes was also used. The microelectrodes were placed in a boiling 3 M KCl solution which was further subjected to rigorous boiling for about 20 minutes under a reduced pressure in a dessicator. This method of filling the microelectrodes has its advantage, since the microelectrodes can be used shortly after filling.

The resistance of the microelectrodes was measured with the help of an electronic ohmmeter (Elco Electronic Instrument Co. Inc., Long Island City, New York). Only those electrodes having resistances between 10 to 30 MΩ were used in the experiments. The most satisfactory results were obtained with stimulating microelectrodes having resistances around 10 MΩ. The tip junction potential (tip potential) of the microelectrodes was not measured because such a source of error would not affect the conclusions.

(b) Recording System: Each microelectrode was operated by a separate micromanipulator. The electrode was held to a plastic arm

in the manipulator by the chlorided silver wire used to connect the electrode to the cathode-follower. A small piece of rubber tubing around the wire fitted into the shaft of the electrode and held the electrode firmly onto the wire. The reference electrodes consisted of chlorided silver plates. The arrangement of stimulating and recording electrodes assembly is shown in the diagram of Figure 4. Current and potential across the fibre membrane were recorded by separate channels using conventional type differential D.C. amplifiers and were displayed on a dual beam cathode-ray oscilloscope. The current intensity was calculated from the potential difference across a 150 KΩ series resistor. For calibration of the voltage amplifier, known voltages were introduced between this resistor and the ground, while the grid of the other side was grounded. The grid current of the input tube was occasionally checked and in most cases was found in the order of 1×10^{-11} A, an amount too small to have introduced any measurable errors.

The most difficult technique to master was the insertion of two microelectrodes inside the same cell. The distance between the two electrodes was nearly 50 μ. An eye-piece micrometer was placed in one of the oculars of the dissecting microscope for measuring the distance between the electrode tips. The stimulating electrode was inserted into a fibre when the switch was in position 2 (see Figure 4). A successful penetration was indicated by the usual sudden appearance of the resting potential. The switch was then returned to position 1 and the recording electrode was implanted. That both electrodes were

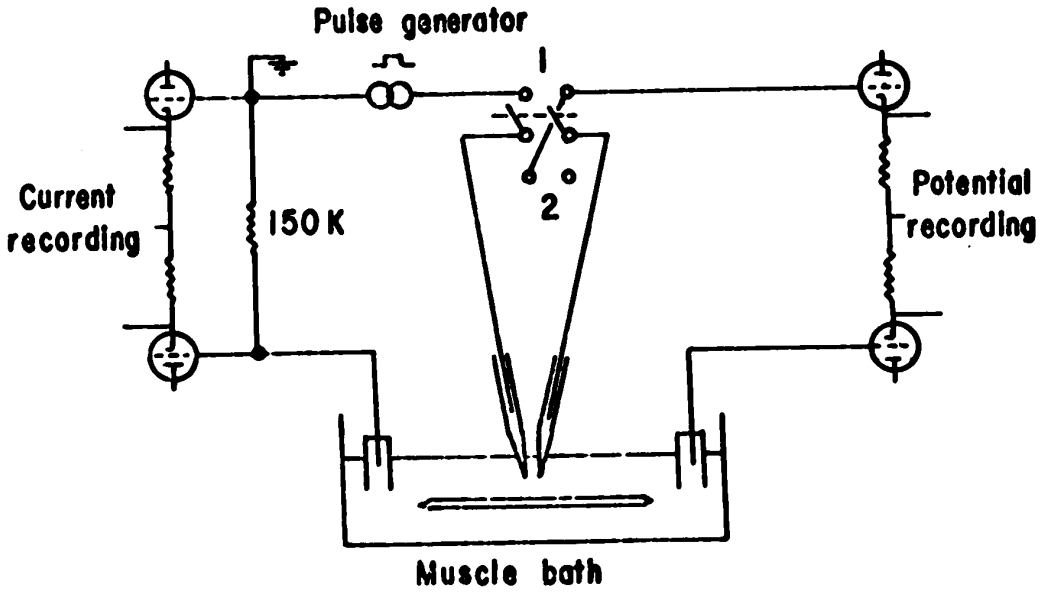

Figure 4. Arrangement for measuring current and potential across the fibre membrane with two intracellular electrodes. Note double-pole, double-throw switch, position 2 for inserting stimulating electrode, position 1 for testing electrode insertion and for normal use.

in the same cell was checked by applying 2 msec hyperpolarizing pulses (tip negative) with the stimulating electrode and recording the presence of an electrotonic potential with the recording electrode.

In order to determine the current-voltage relation, several hyperpolarizing and depolarizing pulses of 2 msec duration and varying strengths were applied. The responses were recorded by a camera from a dual beam oscilloscope slaved to the monitor oscilloscope. For measuring the membrane time constant however, hyperpolarizing square-pulses of 70 msec duration were used. A more detailed discussion of this technique can be found elsewhere (Nastuk and Hodgkin, 1950; Fatt and Katz, 1951, 1953; Frank, 1958).

CHAPTER IV

RESULTS

CHAPTER IV. RESULTS

(A) General Comment:

Since nine different drugs have been used in this study and in each case a similar experimental procedure and recording arrangement has been employed, a detailed description of the results and data analysis procedure will be provided for one drug only. Each drug will, of course, be dealt with separately and the effects produced by each drug on the various electrical properties studied will be described briefly. Such an approach has been felt necessary to avoid repetition and confusion. The drugs used have been listed previously. Definitions of some of the terms used in the description of results are given below.

(B) Definitions:

a) Excitability: The term excitability is used to refer to the reciprocal or inverse of threshold. From threshold is meant the strength of the smallest amount of current needed to produce a measurable action potential.

b) Threshold Depolarization: Threshold depolarization is defined as the depolarization at the end of the 2 msec current pulse which is just sufficient to initiate an action potential which begins at the end, or just after the end of the stimulus.

c) Threshold Current: Threshold current refers to the amount of current required to initiate an action potential.

d) Critical Level of Membrane Potential: Critical level of membrane potential is the "firing level" - that is, the membrane potential at which the action potential is initiated. It is not measured directly, but calculated as the difference between resting membrane potential and the threshold depolarization.

e) Overshoot Potential: Overshoot potential or the so-called reversal potential will be used to designate the potential difference across the fibre membrane at the peak of the action potential. The overshoot is considered to be positive when the potential inside the fibre is positive with respect to the bathing solution.

f) Effective Membrane Resistance: The term effective membrane resistance or the input resistance refers to the resistance between the inside and the outside of the fibre membrane at the point of stimulation. It is assumed that the distance between the stimulating and the recording electrodes is zero.

(C) CHLORPROMAZINE:

i. Studies with Extracellular Stimulation. Preliminary experiments indicated that when the muscle strips were soaked in Ringer's solution containing chlorpromazine, only a partial recovery of the excitability and the compound action potential was possible even after repeated washings with Ringer's solutions up to a period of 3 to 4 hours. For instance, 20 to 30% recovery of the compound action potential and about 30 to 35% recovery of the excitability was achieved after the muscle had been exposed to 10×10^{-6} g/ml of chlorpromazine for 30 minutes. When greater concentrations of chlorpromazine than this were used, these responses were even more slowly and less fully reversed upon the removal of the drug. The conclusion drawn from these studies was that there is a loosely bound portion of the chlorpromazine which can be washed off with relative ease, whereas the rest of the drug remains in the tissue and continues to exert its effect for a considerable period of time. It is also possible that chlorpromazine might have produced a non-specific toxic effect which was only partially reversible. It was therefore decided that in order to determine the concentration-effect relationship with chlorpromazine, the drug concentration applied should be kept constant in each preparation, and only the time interval for exposure should be varied. The following procedure was used. The isolated muscle strip was allowed to equilibrate in normal Ringer's solution for at least 60 minutes before the control measurements were started. Usually 3 to 4 control responses of the excitability and the maximum amplitude of

the compound action potential in Ringer's were obtained, recordings being done at 10 minutes intervals. The mean of these control responses of each muscle preparation was used to calculate the percent change in the excitability and in the size of the compound action potential after the drug treatment. After the control recordings the muscle was placed into the specified concentration of chlorpromazine and only one concentration was tested on any one preparation. With low concentrations of chlorpromazine (0.25 to 5 x 10^{-6} g/ml), the measurements were made every 30 minutes up to 180 minutes. As the chlorpromazine concentrations increased the recording interval was reduced to 10 to 15 minutes.

The results of all the experiments of the time course study of the chlorpromazine-induced effect on the amplitude of the compound action potential and on the excitability are described in Figure 5-A and B respectively. It can be seen that the size of the action potential and the excitability remain essentially unaffected with 0.25 x 10^{-6} g/ml of chlorpromazine. However, a progressive depression of these two measured parameters is noticed with increasing concentrations of chlorpromazine. The action potential production is virtually abolished with 50 x 10^{-6} g/ml of chlorpromazine after 30 minutes exposure (Figure 5-A), and the excitability is reduced to about 11.5 $\pm$ 2.5% as compared with the control (Figure 5-B). On the other hand, treating the muscle with 2.5 x 10^{-6} g/ml chlorpromazine for 180 minutes the amplitude of the compound action potential is decreased to about 44.6 $\pm$ 5.2% (Figure 5-A), and the excitability declines to about 36.3

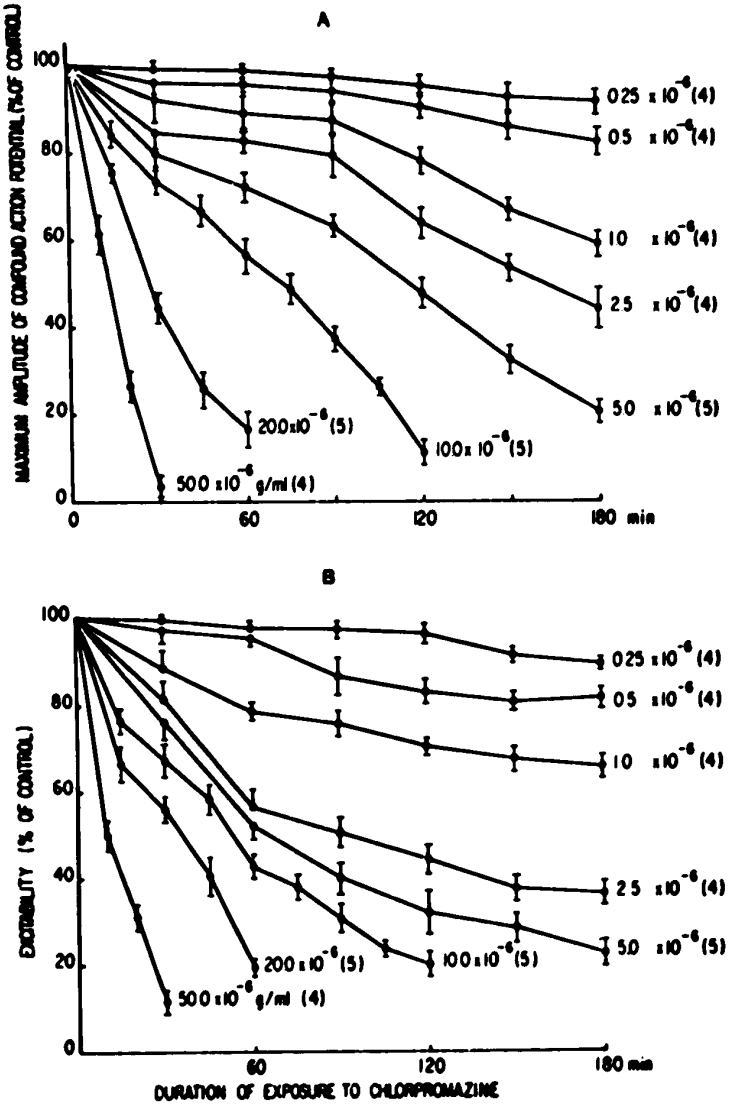

Figure 5. Effects of various concentrations of chlorpromazine on (A) the maximum size of the compound action potential, and on (B) the excitability, calculated as the inverse of the threshold current in frog sartorius muscle strips. Figures in parentheses represent the number of muscle strips used, the vertical bars indicate ± S.E. of the mean.

± 3.1% (Figure 5-B) in comparison with the control values. These results show that there seems to exist an inverse relation between the soaking interval and the chlorpromazine concentration in the bathing medium so far as the depressant actions of this drug on the excitability and on the height of the compound action potential of the frog skeletal muscle are concerned. Figure 5-A and B also show that with chlorpromazine concentrations ranging from 0.5 to 2.5 x 10^{-6} g/ml, the equilibrium (steady state) between the muscle membrane and the chlorpromazine containing Ringer's seems to be reached after about 120 minutes, because no appreciable further depression in the compound action potential amplitude and in the excitability occurs afterwards up to 180 minutes.

Since in a chlorpromazine treated muscle the excitability is decreased (threshold increased), and the size of the compound action potential is either reduced or the action potential production is completely blocked, it seems possible that such depressant effects are invoked by inhibiting the specific increase of sodium conductivity which normally follows an adequate stimulus. Such depressant effects of chlorpromazine are qualitatively similar to those previously reported for procaine (Shanes, 1958; Taylor, 1959; Inoue and Frank, 1962), for diethyl ether (Inoue and Frank, 1965), for hexafluordiethylether (Inoue *et al.*, 1967) and for ethyl alcohol (Inoue and Frank, 1967), and thus provide a basis for comparison with results obtained in the present study by using intracellular microelectrodes under similar experimental conditions.

ii. Studies with Intracellular Electrodes: Two microelectrodes were used in these experiments, one for passing 2 msec depolarizing (stimulating) square-pulses through the membrane and the other to measure potential across the membrane. Figure 6 illustrates the typical action potentials recorded from single muscle fibres, and also several other general observations made in the course of this investigation. The upper line represents the zero potential level (electrode outside the fibre) and the lower line the potential when the microelectrode was inside the fibre. It can be seen from Table I that the resting membrane potential remain essentially unaltered by the chlorpromazine concentrations tried. The overshoot of the action potential is decreased by increasing concentrations of chlorpromazine and is completely abolished by 5×10^{-6} g/ml chlorpromazine (Figure 6-C_1). It can also be seen from Figure 6 and Table I that the "firing level" or the "critical level of the membrane potential" (previously defined as the difference between the resting membrane potential and the threshold depolarization) is slightly decreased with increasing concentrations of chlorpromazine.

Some membrane electric constant measurements obtained with intracellular microelectrodes are summarized in Table I. Chlorpromazine treatment produces an increase in the threshold depolarization and in the threshold current needed to initiate an action potential. A slight but not a significant increase in the threshold current as well as the threshold depolarization is observed by treating the muscle in 1×10^{-6} g/ml of chlorpromazine. However, when the muscle is immersed

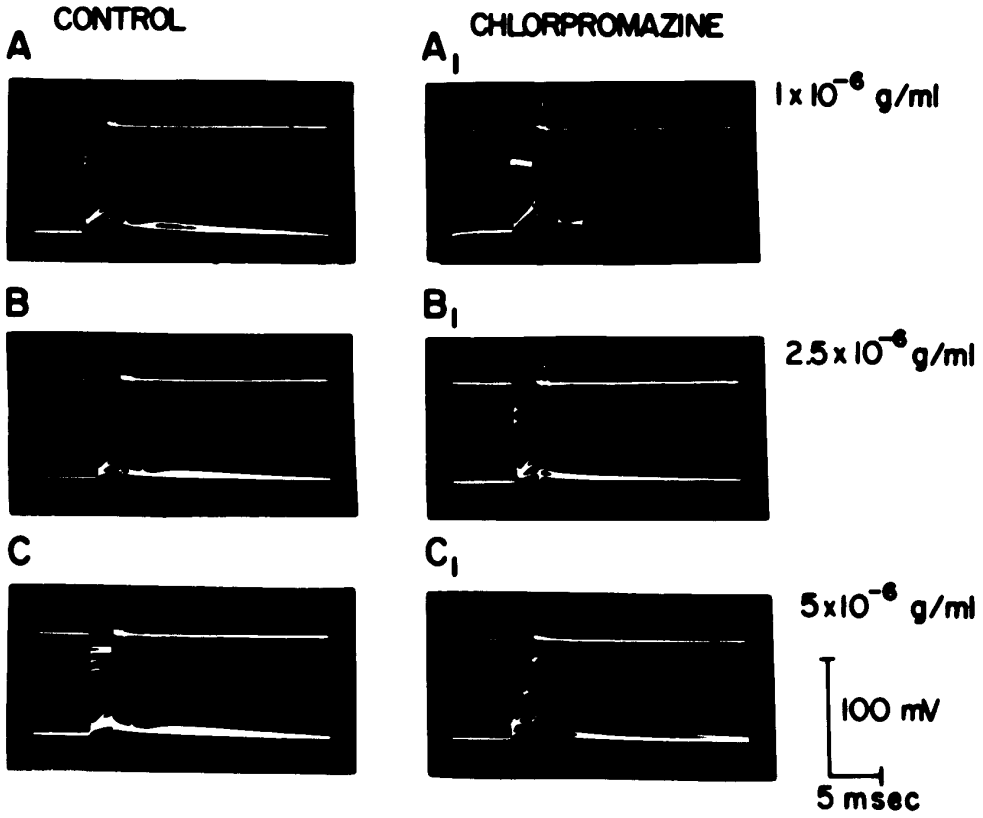

Figure 6. Effect of chlorpromazine on the intracellularly recorded action potentials from the frog sartorius muscle fibres. A, B, and C, are the control records from three separate muscles in Ringer's solution. A_1, B_1, and C_1, are the responses recorded after 180 minutes exposure to chlorpromazine. Upper traces represent stimulus current records; lower traces show the passive electrotonic depolarizations and the action potentials produced by 2 msec current pulses.

TABLE I

Effects of chlorpromazine on some membrane electrical properties of frog sartorius muscle fibres. The muscles were exposed to the specified concentration of chlorpromazine for 3 hours before the measurements were obtained.

Muscles (No.)	Fibres (No.)	Chlorpromazine Concentration (X 10^{-6} g/ml)	Resting Potential* (mV)	Threshold Depolarization (mV)	Threshold Current (X 10^{-7} Amp.)	Effective Resistance (K.Ω)	τ_m (msec)
3	39	0	89.5 ± 1.6	24.1 ± 1.1	1.4 ± 0.65	255 ± 8	11.6 ± 0.4
3	28	1.0	86.6 ± 2.2	27.4 ± 1.4	1.8 ± 0.18	259 ± 11	12.1 ± 0.3
4	42	0	87.7 ± 1.8	24.1 ± 0.7	1.5 ± 0.08	258 ± 12	11.8 ± 0.5
4	25	2.5	90.3 ± 1.5	34.8 ± 1.2 **	2.2 ± 0.11	260 ± 9	12.8 ± 0.8
3	32	0	88.8 ± 1.3	21.8 ± 1.6	1.7 ± 0.04	262 ± 13	13.3 ± 0.6
3	21	5.0	89.2 ± 2.1	41.2 ± 2.7 **	3.1 ± 0.22 **	268 ± 10	14.6 ± 0.8

* Mean ± S.E., derived from mean values for each muscle.

** Treatment means are significantly different from the control means ($P < 0.05$).

in Ringer's having 5 x 10^{-6} g/ml of chlorpromazine, the threshold depolarization is raised from 21.8 $\pm$ 1.6 to 41.2 $\pm$ 2.7 mV, representing an increase to about 2 times the control value. Concurrently the threshold current is increased from 1.7 $\pm$ 0.04 to 3.1 $\pm$ 0.22 x 10^{-7} amperes, which indicates an approximate increase of about 1.8 times above the original values. The effects produced by chlorpromazine on the isolated frog skeletal muscle in the present study are comparable to those obtained with a wide variety of central nervous system depressant agents as reported previously by a number of investigators (Bishop, 1932; Straub, 1956a; Thesleff, 1956; Inoue and Frank, 1962, 1965, 1967; Frank and Sanders, 1963; Frank and Pinsky, 1966; Hille, 1966; Jhamandas, 1969). The data obtained in the present study suggests that the increase in threshold depolarization and the increase in the threshold current (depression of excitability) by chlorpromazine treatment cannot be assigned to the depolarization of the fibre membrane, but is most likely due to the inhibition of the specific increase in the sodium conductivity which is normally associated with membrane depolarization and the action potential production.

iii. Effect on Current-Voltage Relation: When a square-wave pulse of polarizing current was applied to a muscle fibre through one of the inserted microelectrodes, an electrotonic potential with slowly rising and falling phases developed across the fibre membrane. This was recorded through the other microelectrode. When weak currents were employed, the depolarizing (catelectrotonic) and hyperpolarizing

(anelectrotonic) potentials induced by equal current pulses of opposite polarity were of equal size. However, as the current intensity was increased, the depolarizing potentials became progressively greater than the hyperpolarizing potentials induced by current pulses of equal strength. This excess in the size of the depolarizing potential has been called by Hodgkin (1938) as the "local response."

Figure 7-A, B and C represent typical plots of the relation between applied current and generated potential of the fibres soaked in Ringer's solution; and Figure 7-A_1, B_1, and C_1 show the corresponding counterparts after the specified chlorpromazine treatment. The potentials plotted were recorded at the end of 2 msec current pulses, only one concentration of chlorpromazine was tested on any one preparation. Although several fibres were impaled from each muscle, only the data of five fibres at random were selected to construct the graphs in each case. Such a selection was felt necessary to prevent excessive overlapping of different symbols used to represent individual muscle fibres in every graph. Figure 7-A, B, and C indicate that there is a linear relation, passing through the origin, between the current and the change in the membrane potential evoked. However, a deviation from linearity (the local response) appears as the intensity of the depolarizing current is increased. In each case the slope was calculated from the data obtained after passing hyperpolarizing pulses through the fibres in Ringer's solution without chlorpromazine (Figure 7-A, B and C). The same linear regression line passing through the origin in the corresponding graph was drawn to fit the data procured

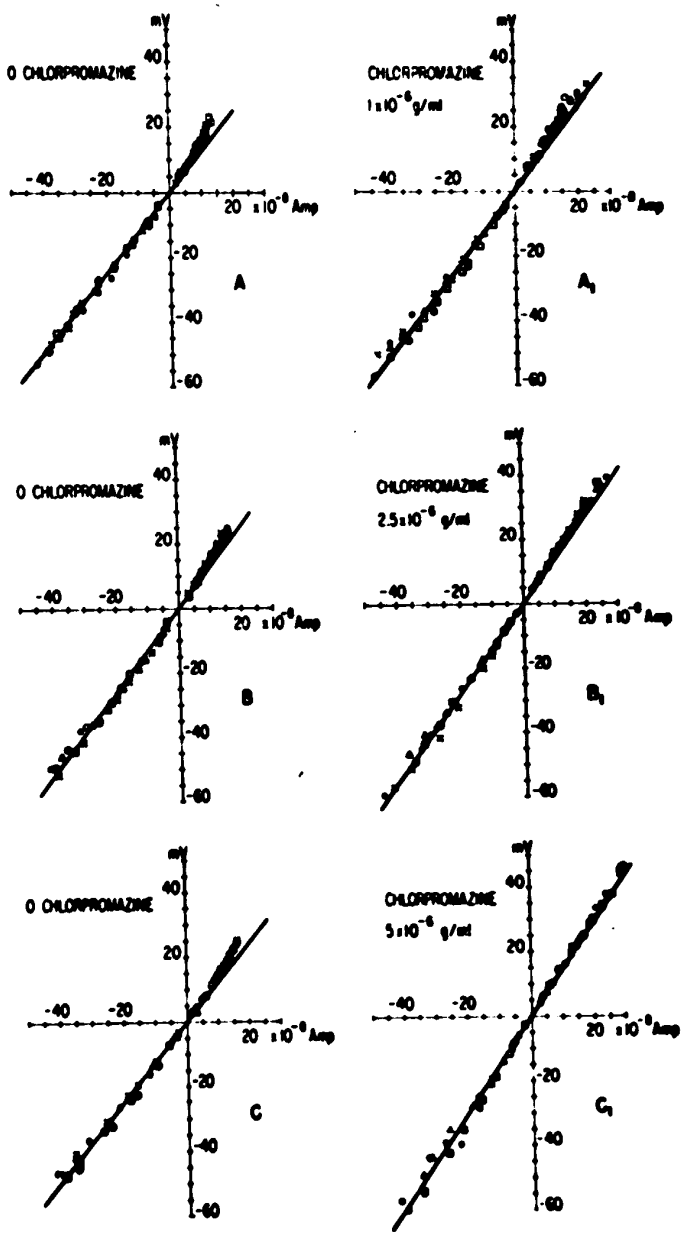

Figure 7. Effects of chlorpromazine treatment on the current-voltage relation of frog sartorius muscle fibres. The different symbols on each graph represent individual muscle fibres. A, B, and C, are the control responses obtained from three separate muscles in Ringer's solution. A_1, B_1, and C_1, are the responses recorded after exposure to chlorpromazine for 180 minutes. For each muscle, the same linear regression line is drawn to fit the data obtained at the specified concentration. The potentials were measured at the end of 2 msec current pulses of the specified intensity.

after each drug treatment. It can be noticed (Figure 7-A_1, B_1, and C_1) that chlorpromazine does not alter the slope of this linear relation, from which it can be concluded that the passive membrane conductance remains unchanged. The additional observation (Table I) that there is no significant difference in the membrane time constant (τ_m), and the effective membrane resistance between the inside and the outside of the fibres at the point of stimulation also indicate that the passive membrane conductance remains practically unaltered after the chlorpromazine treatment.

The largest depolarizations recorded on all the graphs are the threshold depolarizations. The most obvious change produced by chlorpromazine treatment (Figure 7-A_1, B_1, and C_1) is a suppression of the "local response." At a concentration of 5×10^{-6} g/ml chlorpromazine a few of the muscle fibres became completely inexcitable. For the muscles where the threshold was increased the voltage-current relationship continued to fit the linear regression line above the previous threshold points (Figure 7-C_1). Such an effect suggests that chlorpromazine depresses or blocks excitability by inhibiting the specific increase in membrane sodium conductivity which is responsible for "local response" and the rising phase of the action potential. Inoue and Frank (1962) have reported qualitatively similar effects of procaine treatment on the current-voltage relation of frog sartorius muscle fibres.

iv. Effect on the Maximum Rate of Rise of the Action Potential: In addition to being the underlying cause of the "local response" the increase in membrane sodium conductance which follows an adequate depolarization of the fibre membrane also is a major factor in determining the rate of rise of the action potential and the maximum amplitude of the action potential (Hodgkin, 1951; Shanes, 1958). For this reason, the effects of drug treatments on these two parameters were investigated. In the analysis of these results, two relations pointed out by Hodgkin and Katz (1949, Equations 3 and 8.1) were especially useful. These are: 1) the maximum rate of change of the membrane potential is proportional to the net inward current due to the transfer of sodium ions from the outside to the inside of the fibre, and 2) at this point the sodium current is proportional to sodium permeability of the fibre and the difference in the internal and external sodium concentrations. Since at this time the net inward current is mainly due to inward transfer of sodium ions, the further assumption has been made here that the maximum rate of rise of the action potential is proportional to the sodium permeability of the membrane during the rising phase of the action potential.

It was pointed out earlier that studies with extracellular electrode technique showed that when chlorpromazine was used in a concentration of 2.5×10^{-6} g/ml, the equilibrium between the medium surrounding the muscle strip and the cell membrane was reached in about 120 minutes and after that no significant depression in the amplitude of the compound action potential and that of the excitab-

ility was noticed up to 180 minutes. Keeping in view these observations it was decided that experimental recordings should be made only after 120 minutes exposure to chlorpromazine. Control recordings of normal and differentiated action potentials were made in Ringer's with normal (114 mM), low (57 mM) and high (171 mM) sodium before exposing the tissue to chlorpromazine. After 120 minutes in normal Ringer's and 2.5×10^{-6} g/ml chlorpromazine the first experimental recordings were made. The muscle was then soaked in Ringer's containing 57 mM - Na^+ and 2.5×10^{-6} g/ml of chlorpromazine for 30 minutes before further records were obtained. Finally, the muscle was exposed to Ringer's solution containing the usual amount of chlorpromazine and 171 mM - Na^+ and the action potentials were again recorded after 30 minutes. Typical recordings of differentiated action potentials are shown in Figure 8. It can be noticed that treating the muscles with chlorpromazine had an effect similar to that of reducing the external Na^+ concentration.

The quantitative data obtained from several fibres in four muscles before and after the treatment with chlorpromazine (2.5×10^{-6} g/ml) and various external sodium concentrations on the maximum rate of rise of the action potentials are plotted in Figure 9. Interpreted in the light of the arguments presented earlier, these results suggest a competitive antagonism between the chlorpromazine and the sodium ions. The effect of 2.5×10^{-6} g/ml of chlorpromazine contained in normal Ringer's (114 mM - Na^+) being approximately equivalent to a 50% reduction in the extracellular Na^+ concentration. Figure 9 illustrates that the inhibitory action of chlorpromazine could be overcome by raising

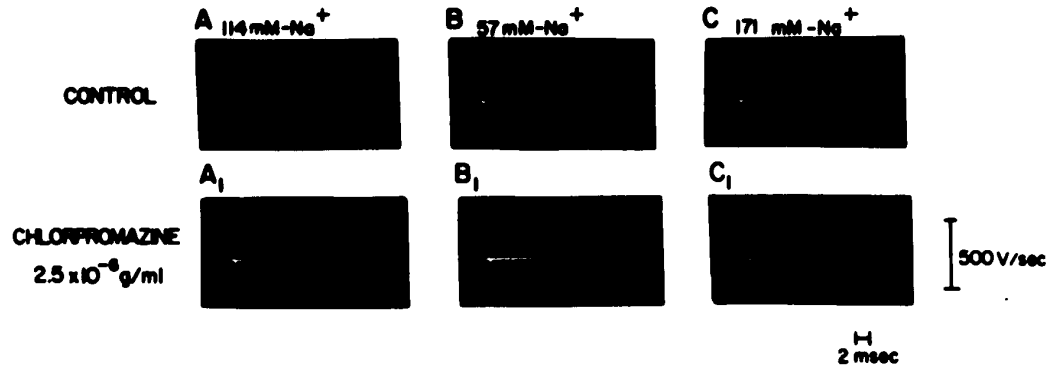

Figure 8. Differentiated, intracellularly recorded action potentials showing the effect of chlorpromazine on the maximum rate of rise of the action potential. The fibres were stimulated with an extracellular pore electrode at a point distant from the recording electrode. The stimulus artifact appears at the start of each record.

The peak of each record represents the maximum rate of rise of the action potential under the specified condition. Responses were obtained from different fibres of the same muscle. Sodium concentration of the bathing solution is shown above the records. The control records (chlorpromazine = 0) with low and high sodium concentration were obtained after 30 minutes soaking in each case. The muscle was exposed to normal Ringer's solution containing chlorpromazine up to 120 minutes, and for at least 30 minutes in low and high sodium Ringer's containing chlorpromazine before each recording. A, B, and C, without chlorpromazine; A_1, B_1, and C_1, 2.5 x 10^{-6} g/ml of chlorpromazine.

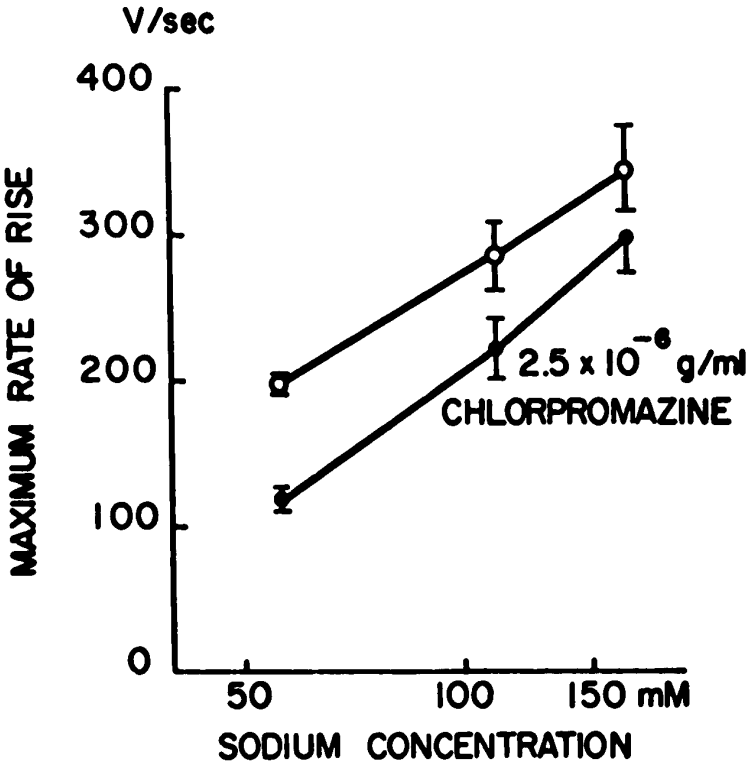

Figure 9. Effect of extracellular sodium concentration and of chlorpromazine on the maximum rate of rise of intracellularly recorded action potentials. Upper curve, Ringer's solution without chlorpromazine; lower curve, with chlorpromazine (2.5×10^{-6} g/ml). Mean and standard error derived from the mean values obtained in each of four separate preparations. Ordinate, maximum rate of rise of the action potential (V/sec); abscissa, sodium concentration of the bathing medium (mM).

the Na^+ concentration in the external medium.

Exposure to chlorpromazine reduced the size of the overshoot potential and addition of excess sodium (171 mM instead of 114 mM) to the extracellular fluid readily antagonized such an effect (Figure 10). These results also indicate a competitive antagonism between chlorpromazine and external sodium ions. A similar sort of competition between procaine and extracellular sodium ions has been reported in the frog sciatic nerve by Condouris (1961), and in the frog sartorius muscle by Inoue and Frank (1962).

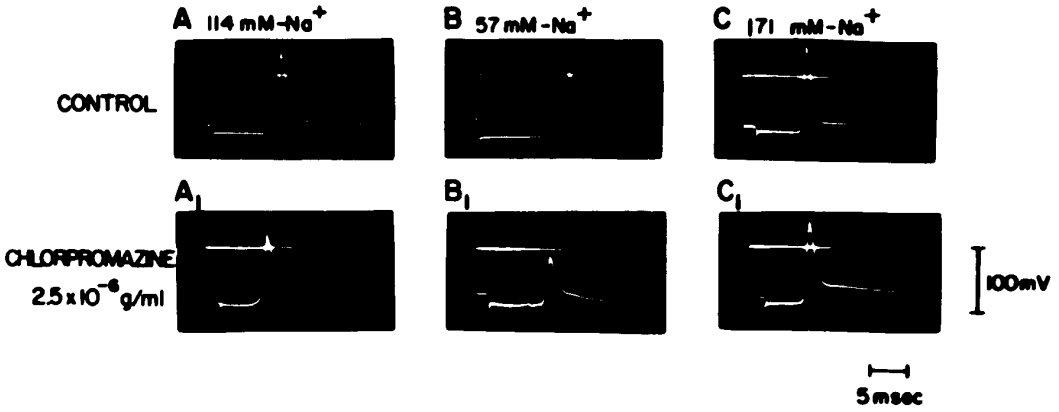

Figure 10. Effect of chlorpromazine on the overshoot of the intracellularly recorded action potentials. Sodium concentration of the bathing medium is indicated above the records. Groups of fibres were stimulated with an extracellular pore electrode kept at some distance apart from the recording electrode. The stimulus artifact appears at the start of each record. The horizontal line across the upper part of all the records represents zero potential. Time and voltage calibrations as indicated.

Responses were obtained from different fibres of the same muscle. The control records (chlorpromazine = 0) with low and high sodium were taken after 30 minutes soaking in each case. The muscle was exposed to normal Ringer's containing chlorpromazine for 120 minutes, and for 30 minutes respectively in low and high sodium Ringer's containing chlorpromazine before each recording. A, B, and C, without chlorpromazine; A_1, B_1, and C_1, 2.5×10^{-6} g/ml of chlorpromazine. In this muscle the mean overshoot potential (mV $\pm$ S.E.) under the various conditions was: A, 27.7 $\pm$ 1.5 (10 fibres); B, 11.3 $\pm$ 1.4 (9 fibres); C, 36.2 $\pm$ 1.3 (11 fibres); A_1, 19.5 $\pm$ 1.2 (8 fibres); B_1, 1.6 $\pm$ 1.9 (7 fibres); and C_1, 25.5 $\pm$ 1.8 (8 fibres).

(D) PROMETHAZINE:

i. Studies with Extracellular Stimulation. Like chlorpromazine, preliminary tests showed that only a partial recovery of the excitability and the compound action potential was possible after the muscle strip had been treated with promethazine. For example, when the muscle was soaked for 30 minutes in 10×10^{-6} g/ml of promethazine in Ringer's, a 30 to 45% recovery of the compound action potential and around 25 to 40% recovery of the excitability was achieved as compared with the original responses after repeated washings in Ringer's up to 2.5 to 3 hours. The per cent reversal of these responses was even less marked when higher concentrations of promethazine were utilized. The probable reasons for the partial recovery were given previously under the description of the results of chlorpromazine. A time course study of the effects of various concentrations of promethazine on the size of the compound action potential and on the excitability was therefore warranted. As mentioned in case of chlorpromazine, after the consecutive recording of 3 to 4 control responses in Ringer's solution the muscle strip was placed into the specified concentration of promethazine. Only one concentration was tested on any one preparation. Recordings were made after every 15 to 30 minutes and were continued up to 45 minutes to 5 hours depending upon the promethazine concentration. Figure 11-A and B illustrate the results of all experiments with different amounts of promethazine on the size of the compound action potential and on the excitability respectively. The amplitude of the compound action potential is scarcely affected by 0.5×10^{-6}

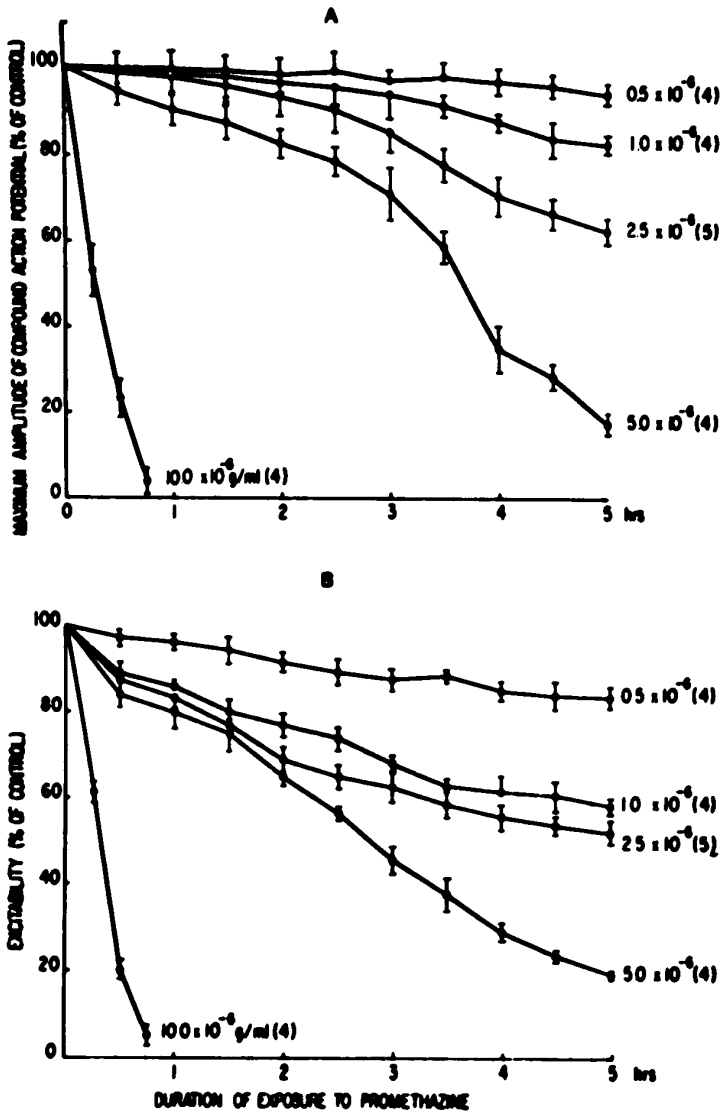

Figure 11. Effects of various concentrations of promethazine on (A) the maximum size of the compound action potential of frog sartorius muscles, and on (B) the excitability, calculated as the inverse of the threshold current. Figures in parentheses indicate the number of muscle strips used, the vertical bars represent ± S.E. of the mean.

g/ml promethazine (Figure 11-A), however the excitability is reduced by about 15% as against the control after a 5 hour period (Figure 11-B), which means that most fibres in the muscle bundle are still excitable. With higher concentrations of promethazine, a progressive decline in the excitability and in the amplitude of the action potential is observed. The action potentials are almost completely blocked when the fibres are left for 45 minutes in 10×10^{-6} g/ml promethazine (Figure 11-A), and the excitability is reduced by about 95% as compared with the control values (Figure 11-B).

Inspection of Figure 11-A and B would indicate that with promethazine concentrations ranging from 0.5 to 2.5×10^{-6} g/ml the equilibrium between the bathing solution and the muscle membrane seems to be reached after about 3.5 to 4 hours, since no further significant change in the size of the compound action potential and in the excitability is observed afterwards up to 5 hours. In general, the effects produced by promethazine on the maximum size of the compound action potential and on the excitability of the frog skeletal muscle are qualitatively very similar to those obtained with chlorpromazine by using the extracellular stimulation technique.

ii. Studies with Intracellular Electrodes. As described before, the standard procedure was to use two microelectrodes, one for stimulation and the other to record the potential change across the membrane. In each series of experiments the depolarizing current strength was increased in a stepwise fashion till the action potential

was generated. Promethazine increased the threshold depolarization and the threshold current in a concentration-dependent manner as shown by typical action potentials in Figure 12. Increasing concentrations of promethazine decreased the firing level or the critical level of the membrane potential. The overshoot of the action potential was reduced with 1.0 and 2.5 x 10^{-6} g/ml promethazine (Figure 12-A_1 and B_1), and was completely abolished with 5 x 10^{-6} g/ml promethazine (Figure 12-C_1).

Some of the membrane electric constants determined by using intracellular electrodes with and without promethazine are displayed in Table II. It can be seen that promethazine does not affect the resting membrane potential. The threshold depolarization as well as the threshold current stay practically unchanged by 1.0 x 10^{-6} g/ml promethazine. However, a significant increase in both these parameters is noticed when the muscles are soaked into 2.5 x 10^{-6} and 5 x 10^{-6} g/ml of promethazine. It requires roughly twice the amount of current (e.g. 3.1 $\pm$ 0.13 instead of 1.6 $\pm$ 0.07 x 10^{-7} amperes) to initiate an action potential in the fibres treated with 5 x 10^{-6} g/ml promethazine. At the same time the threshold depolarization is raised from 25.5 $\pm$ 1.2 to 46.2 $\pm$ 1.3 mV, indicating an approximate increase of 1.8 times the control values. It was interesting to note that the promethazine-induced effects on the threshold depolarization and threshold current were quantitatively similar to those observed with an equivalent amount of chlorpromazine (5 x 10^{-6} g/ml, Table I). Such effects are not unexpected ones because, apart from their pharmacological similarities,

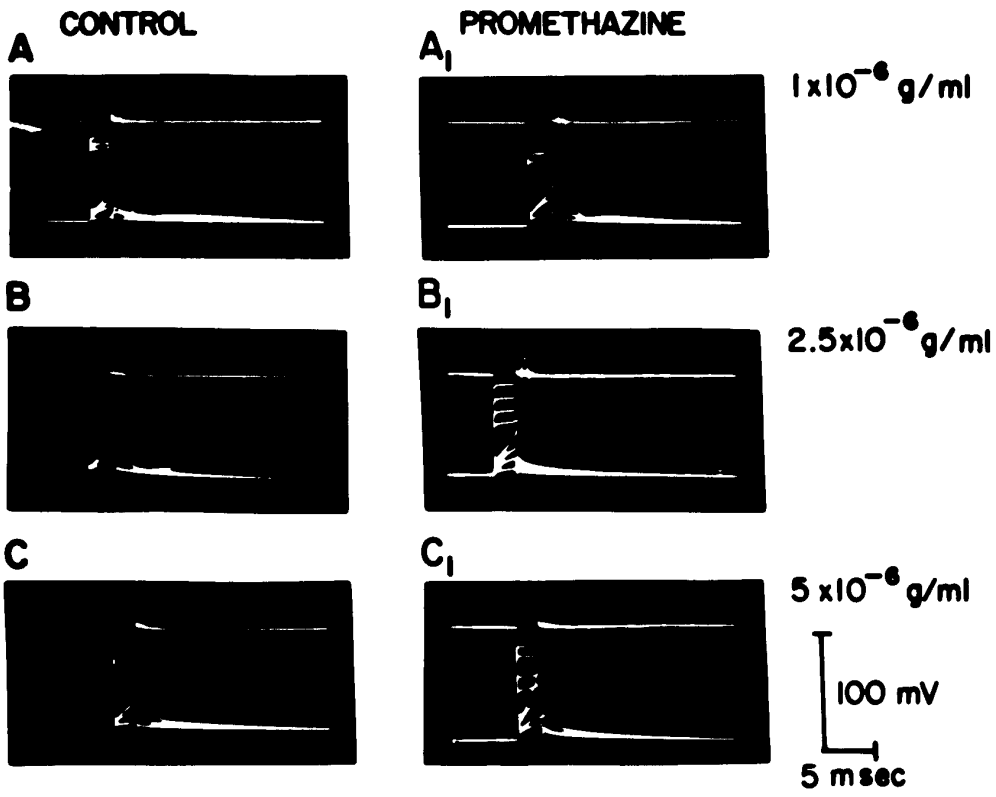

Figure 12. Effect of promethazine on the intracellularly recorded action potentials from the frog sartorius muscle fibres. A, B, and C, are the control responses recorded from three separate muscles. A_1, B_1, and C_1, are the recordings done after 4 hours exposure to a specified concentration of promethazine. Upper traces show stimulus current records; lower traces indicate the passive electrotonic depolarizations and the action potentials produced by 2 msec current pulses.

TABLE II

Effects of promethazine on some membrane electrical properties of frog sartorius muscle fibres. The muscles were exposed to various concentrations of promethazine for 4 hours before the measurements were obtained.

Muscles (No.)	Fibres (No.)	Promethazine Concentration (X 10^{-6} g/ml)	Resting Potential* (mV)	Threshold Depolarization (mV)	Threshold Current (X 10^{-7} Amp.)	Effective Resistance (K.Ω)	τ_m (msec)
2	22	0	91.4 ± 1.4	22.8 ± 0.8	1.6 ± 0.06	255 ± 8	12.4 ± 0.3
	13	1.0	93.3 ± 1.1	24.7 ± 0.9	1.8 ± 0.09	260 ± 10	12.6 ± 0.4
3	32	0	90.7 ± 1.6	24.1 ± 0.5	1.5 ± 0.08	258 ± 5	11.3 ± 0.4
	22	2.5	93.1 ± 1.2	34.9 ± 1.0**	2.5 ± 0.12**	262 ± 12	13.8 ± 0.3
3	24	0	91.8 ± 2.5	25.5 ± 1.2	1.6 ± 0.07	252 ± 7	11.2 ± 0.6
	26	5.0	95.0 ± 1.8	46.2 ± 1.3**	3.1 ± 0.13**	268 ± 6	14.7 ± 0.4**

* Mean ± S.E., derived from mean values for each muscle.

** Treatment means are significantly different from control means ($P < 0.05$).

the chemical and physical properties of these drugs are very close (Jarvik, 1970).

iii. Effect on the Current-Voltage Relation. The basis for studying the effect of depressant drugs on the current-voltage relation was discussed before. Typical examples of relations between applied current and generated potential before and after the promethazine treatment are shown in Figure 13. The potentials plotted were recorded at the end of 2 msec current pulses. Only one concentration of promethazine was tested on any one preparation. Figure 13-A, B, and C represent the graphs plotted from the data of five fibres in each case, obtained from three separate muscles in Ringer's solution without promethazine. In the corresponding counterparts (Figure 13-A_1, B_1, and C_1) the potentials were recorded after soaking the muscles for 4 hours into a specified concentration of promethazine. For each muscle the same regression line passing through the origin as that of the control was drawn to fit the data obtained after the drug treatment. It can be seen that with 1.0×10^{-6} and 2.5×10^{-6} g/ml of promethazine, the slope of the linear relation does not change, from which it can be concluded that the passive membrane conductance is unaltered.

There is a slight increase in the membrane time constant (from 11.2 $\pm$ 0.6 to 14.7 $\pm$ 0.4 msec), and in the effective membrane resistance (from 252 $\pm$ 7 to 268 $\pm$ 6 KΩ) after the muscles are exposed to 5×10^{-6} g/ml of promethazine (Table II). At the same time promethazine (5×10^{-6} g/ml) treatment has a tendency to produce a counter-

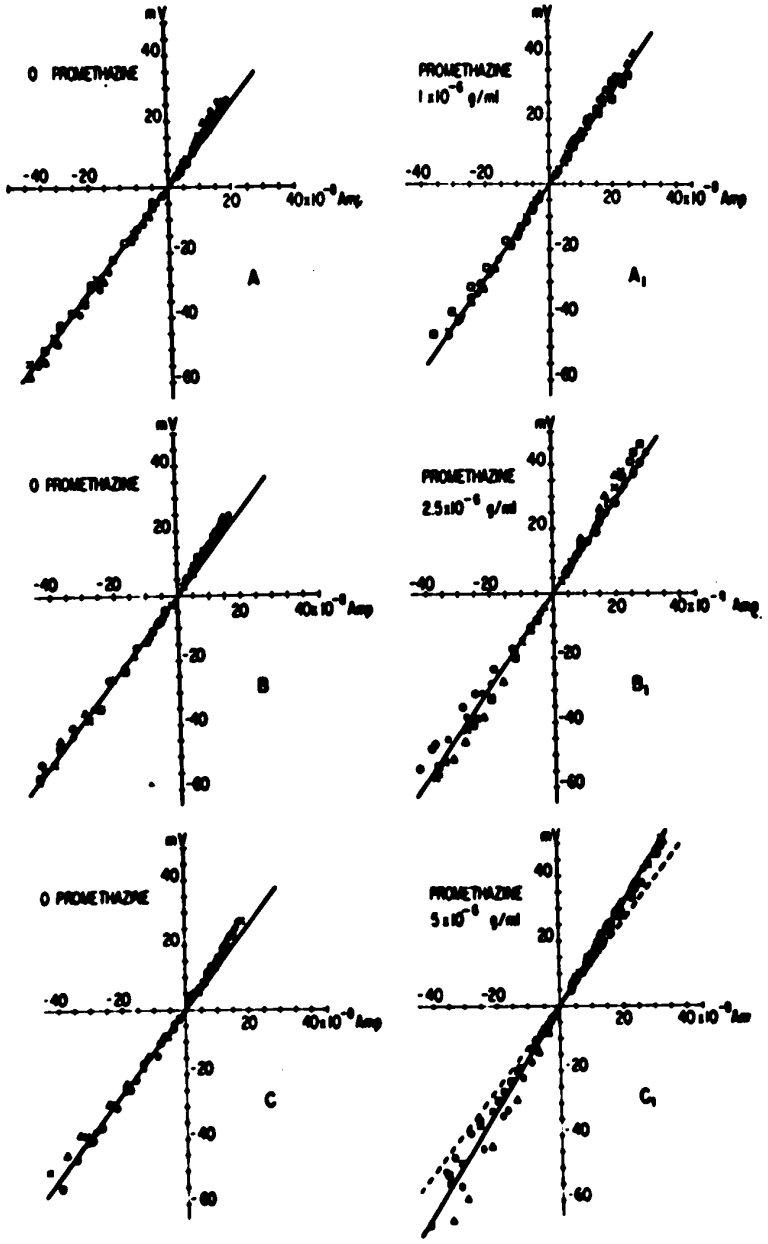

Figure 13. Effects of promethazine treatment on the current-voltage relation of frog sartorius muscle fibres. The different symbols on each graph represent individual muscle fibres. A, B, and C, are the control responses obtained from three separate muscles in Ringer's solution. A_1, B_1, and C_1, are the responses recorded after 4 hours exposure to promethazine. For each muscle, the same linear regression line is drawn to fit the data obtained at the specified concentration. The broken line in C_1 represents the control linear regression line (promethazine = 0) superimposed to show the effect of promethazine treatment. The potentials were measured at the end of 2 msec current pulses of the specified intensity.

clockwise rotation of the current-voltage curve (Figure 13-C_1). It appears that such a rotation is a manifestation of the increased effective membrane resistance between the inside and the outside of the fibres at the point of stimulation.

The largest depolarizations recorded on all the graphs are the threshold depolarizations. The most obvious change produced by promethazine is a suppression of the local response (Figure 13-A_1, B_1, and C_1). In one of the muscles treated with 5×10^{-6} g/ml of promethazine, two fibres out of nine became completely inexcitable (no action potentials were produced) and for those in which the threshold depolarizations were increased the voltage-current measurements also mostly fell on the linear regression line (Figure 13-C_1). All these observations when taken together suggest that the depression or the blockade of membrane excitability produced by promethazine seems to be by virtue of its inhibiting action on the specific increase in membrane sodium conductance which is usually responsible for the local response and the rising phase of the action potential.

iv. Effect on the Maximum Rate of Rise of the Action Potential. Reasons were given previously (p. 17) for the assumption that the maximum rate of rise of the action potential is proportional to the sodium permeability of the membrane during the rising phase of the action potential. Groups of fibres were stimulated with an extracellular pore electrode and the maximum rate of rise was determined by electrical differentiation of action potentials recorded with intracellular elec-

trodes. The procedure followed was precisely similar to that mentioned for chlorpromazine except that, following control recordings the muscle was treated with promethazine (5×10^{-6} g/ml) for 4 hours (bathing solution being changed after every 20 to 30 minutes) before recording experimental action potentials. The muscle was later exposed to 57 mM-Na^+ Ringer's with the same amount of promethazine dissolved for a period of 30 minutes before the records were obtained. Finally, the muscle was kept in 171 mM-Na^+ Ringer's containing promethazine and the action potentials were recorded after 30 minutes. Typical recordings are shown in Figure 14. Treating the fibres with promethazine had an effect similar to reducing the external sodium concentration. However, the reduction of the maximum rate of rise by promethazine was quickly antagonized by increasing the sodium concentration in the bathing medium. The results obtained in four experiments with promethazine and various external sodium concentrations are shown in Figure 15. They suggest a competitive inhibition, with the effect of 5×10^{-6} g/ml promethazine being approximately equivalent to a 50% reduction in the extracellular sodium concentration. In addition, it was found that raising the external sodium concentration to 171 mM would readily increase the size of the overshoot potential previously depressed by 5×10^{-6} g/ml promethazine (Figure 16).

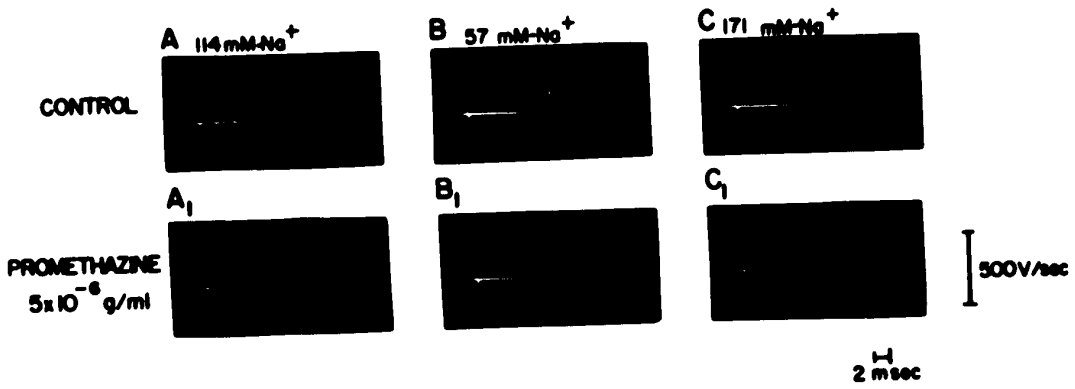

Figure 14. Differentiated, intracellularly recorded action potentials showing the effect of promethazine on the maximum rate of rise of the action potential. The fibres were stimulated with an extracellular pore electrode at a point distant from the recording electrode. The stimulus artifact appears at the start of each record.

The peak of each record represents the maximum rate of rise of the action potential under the specified condition. Responses were obtained from different fibres of the same muscle. Sodium concentration of the bathing solution is shown above the records. The control records with low and high sodium concentration were obtained after 30 minutes soaking in each case. The muscle was exposed to normal Ringer's solution containing promethazine up to 4 hours, and for at least 30 minutes in low and high sodium Ringer's containing promethazine before each recording. A, B, and C, without promethazine; A_1, B_1, and C_1, 5 x 10^{-6} g/ml promethazine.

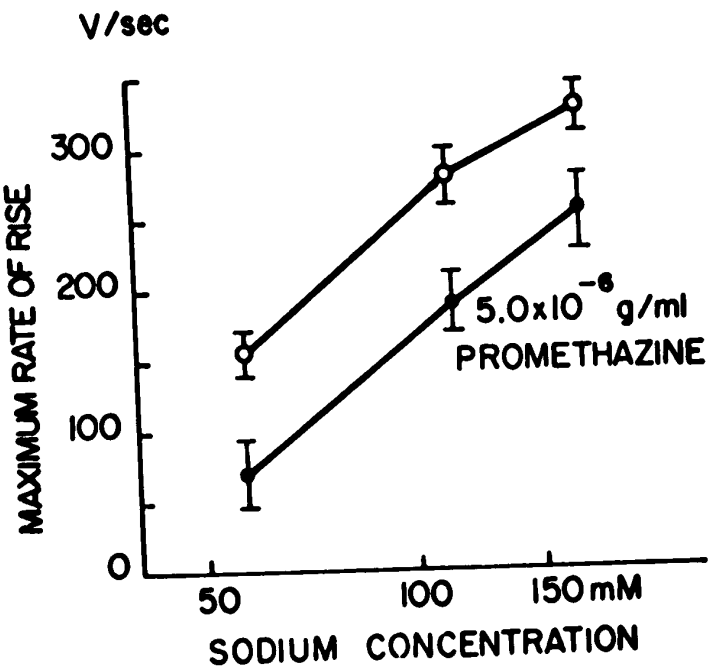

Figure 15. Effect of extracellular sodium concentration and of promethazine on the maximum rate of rise of intracellularly recorded action potentials. Upper curve, Ringer's solution without promethazine; lower curve, with promethazine (5×10^{-6} g/ml). Mean and standard error derived from the mean values obtained in each of four separate preparations. Ordinate, maximum rate of rise of the action potential in V/sec; abscissa, sodium concentration of the bathing medium in mM.

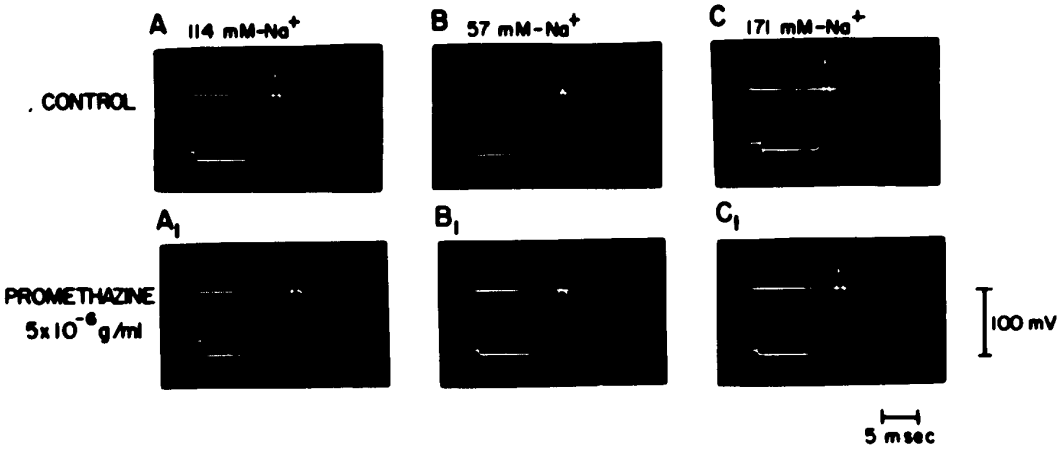

Figure 16. Effect of promethazine on the overshoot of the intracellularly recorded action potentials. Sodium concentration of the bathing medium is indicated above the records. The stimulus artifact appears at the start of each action potential. The horizontal line across the upper part of all the records indicates zero potential.

Action potentials were recorded from different fibres of the same muscle. The control records with low and high sodium were taken after 30 minutes soaking in each case. The muscle was exposed to normal Ringer's solution containing promethazine for 4 hours, and for 30 minutes in high and low sodium Ringer's before each recording. A, B, and C, without promethazine; A_1, B_1, and C_1, 5 x 10^{-6} g/ml promethazine. In this muscle the mean overshoot potential (mV $\pm$ S.E.) under the various conditions was: A, 28.5 $\pm$ 1.4 (11 fibres); B, 13.2 $\pm$ 1.8 (8 fibres); C, 37.6 $\pm$ 1.6 (10 fibres); A_1, 18.2 $\pm$ 1.7 (9 fibres); B_1, 1.8 $\pm$ 2.4 (13 fibres); and C_1, 26.5 $\pm$ 1.8 (11 fibres).

(E) DIPHENHYDRAMINE (BENADRYL):

1. Studies with Extracellular Stimulation. Because preliminary experiments showed an incomplete recovery (for example, after 30 minutes exposure to 20 x 10^{-6} g/ml of diphenhydramine the excitability usually returned to around 70 to 80% and the compound action potential to about 80 to 90% of the original value after two hours washing of the muscle fibres in Ringer's solution), a time course study of the effects of various concentrations of diphenhydramine on the amplitude of the compound action potential and on the excitability was done, as for chlorpromazine. The reasons for the partial recovery were outlined before. After making the control observations, the muscle strip was placed into diphenhydramine containing Ringer's and only one concentration was tested on any given preparation. The measurements were made at 30 minute intervals and were continued for 1 to 4 hours depending upon the concentration being used.

The results of all the concentration-effect relations are shown in Figure 17-A and B. The excitability and the maximum size of the compound action are very slightly affected with 0.25 x 10^{-6} g/ml diphenhydramine. However, a progressive depression of the action potential amplitude and of the excitability is seen with higher concentrations of diphenhydramine. The height of the compound action potential is reduced to about 4 $\pm$ 2.5% (Figure 17-A), and the excitability is decreased to about 9 $\pm$ 3.2% (Figure 17-B) as compared with the control values after soaking the muscle for 60 minutes into 20 x 10^{-6} g/ml diphenhydramine. Figure 17-A and B also show that the steady

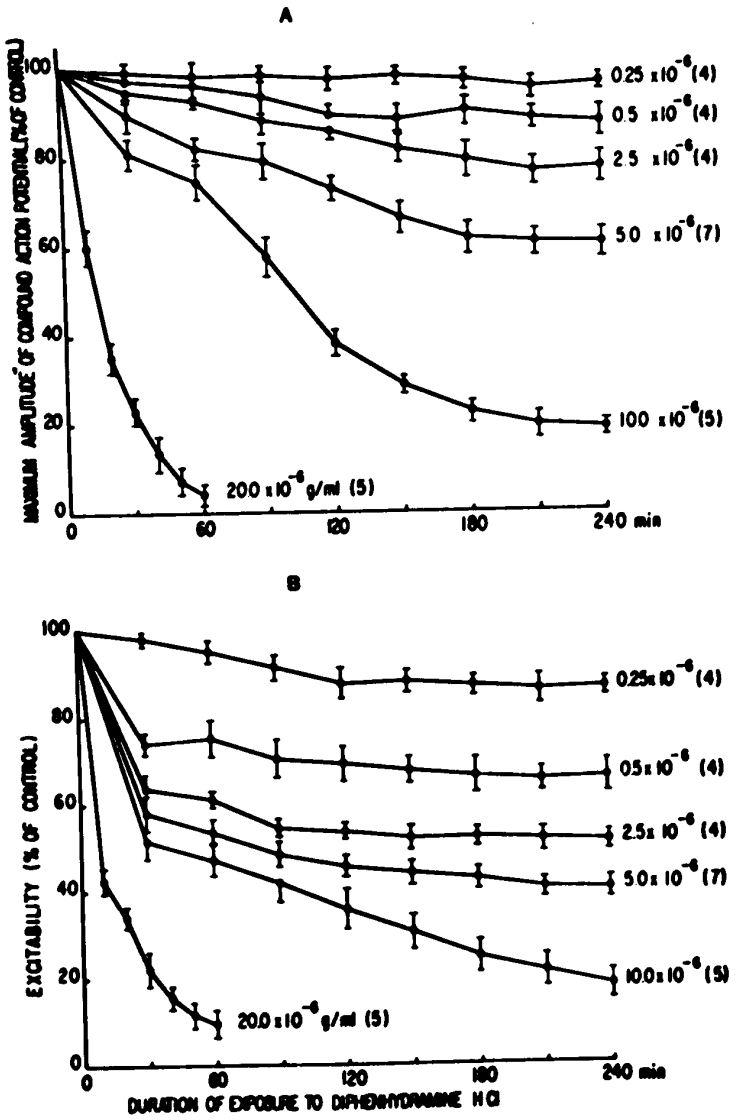

Figure 17. Effects of various concentrations of diphenhydramine on (A) the maximum size of the compound action potential of the frog sartorius muscles, and on (B) the excitability, calculated as the inverse of the threshold current. Figures in parentheses indicate the number of muscle strips used, the vertical bars represent ± S.E. of the mean.

state (equilibrium) between the extracellular medium containing diphenhydramine (0.5 to 10 x 10^{-6} g/ml) and the muscle fibre membrane seems to be reached after about 120 to 150 minutes, because no significant further decrease in the height of the action potential and in the excitability occurs afterwards up to 240 minutes.

ii. Studies with Intracellular Electrodes. Some of the measurements obtained with intracellular microelectrodes are summarized in Table III. It takes practically twice the amount of current (3.2 $\pm$ 0.2 instead of 1.6 $\pm$ 0.03 x 10^{-7} amperes) to initiate an action potential in the fibres previously exposed to 2.0 x 10^{-6} g/ml diphenhydramine. Treating the muscles with 10 and 20 x 10^{-6} g/ml diphenhydramine, the threshold depolarization necessary for the action potential production is changed from a control level of 24.5 $\pm$ 0.5 to 32.0 $\pm$ 1.3 mV and from 24.9 $\pm$ 0.6 to 43.6 $\pm$ 2.0 mV respectively. From these observations it follows that the excitability of the muscle fibre membrane is considerably depressed in the presence of diphenhydramine. No appreciable change in the resting membrane potential is observed.

Figure 18 represents the typical action potentials recorded with two microelectrodes into the same fibre. It can again be seen that diphenhydramine increases the threshold depolarization and the threshold current needed to initiate an action potential, and decreases the firing level. The overshoot of the action potential is completely abolished with 20 x 10^{-6} g/ml diphenhydramine (Figure 18-B_1). Complete blockade of the action potential production was noticed in few of the

TABLE III

Effects of diphenhydramine on some membrane electrical properties of frog sartorius muscle fibres. The muscles were exposed to various concentrations of diphenhydramine for 2 hours before the measurements were obtained.

Muscles (No.)	Fibres (No.)	Diphenhydramine Concentration (X 10^{-6} g/ml)	Resting Potential* (mV)	Threshold Depolarization (mV)	Threshold Current (X 10^{-7} Amp.)	Effective Resistance (K.Ω)	τ_m (msec)
3	18	0	90.8 ± 2.1	24.5 ± 0.5	1.6 ± 0.04	257 ± 9	12.0 ± 0.4
	14	10.0	88.1 ± 1.1	32.0 ± 1.3 **	2.1 ± 0.06 **	283 ± 13 **	15.5 ± 0.6**
4	26	0	89.5 ± 1.2	24.9 ± 0.6	1.6 ± 0.03	262 ± 14	12.4 ± 0.4
	23	20.0	91.3 ± 1.4	43.6 ± 2.0 **	3.2 ± 0.2 **	334 ± 11 **	18.5 ± 0.3**

* Mean ± S.E., derived from mean values for each muscle.

** Treatment means are significantly different from the control means ($P < 0.05$).

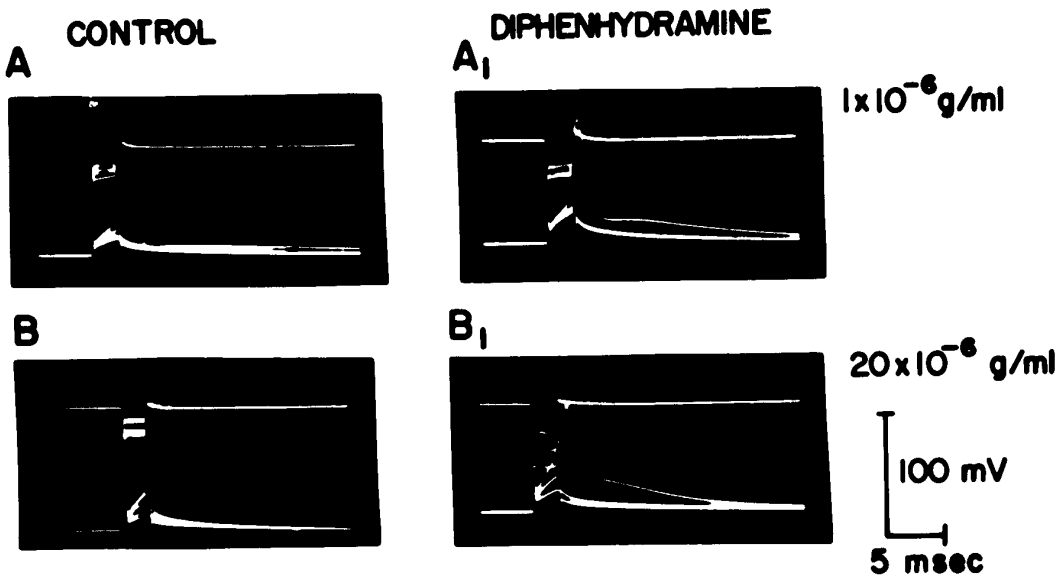

Figure 18. Effect of diphenhydramine on the intracellularly recorded action potentials from the frog sartorius muscle fibres. A, and B, are the control responses recorded from two separate muscles. A_1, B_1, are the recordings done after 2 hours exposure to a specified concentration of diphenhydramine. Upper traces show stimulus current records; lower traces indicate the passive electrotonic depolarizations and the action potentials produced by 2 msec current pulses.

fibres in the presence of 20 x 10^{-6} g/ml diphenhydramine. As in the case of chlorpromazine and promethazine, these effects suggest that diphenhydramine depresses or blocks membrane excitability by inhibiting the specific increase in membrane sodium conductivity which is responsible for the action potential generation.

iii. Effect on the Current-Voltage Relation. The effects of diphenhydramine on current-voltage curves for 2 msec pulses are shown in Figure 19. Diphenhydramine produced a small but definite counterclockwise rotation of the regression lines. The amount of this rotation was about the same with 10 and 20 x 10^{-6} g/ml diphenhydramine. Since diphenhydramine also increased τ_m and the effective membrane resistance, this rotation therefore must have resulted from an increase in the effective membrane resistance. Soaking the muscles into diphenhydramine-Ringer's solution either greatly diminished or completely eliminated the local response (Figure 19-A_1 and B_1). Exposing isolated frog's sartorius muscle to ether also produces an increase in the τ_m, and in the effective membrane resistance (Inoue and Frank, 1965).

iv. Effect on the Maximum Rate of Rise of the Action Potential. Differentiated, intracellularly recorded action potentials showing the effect of diphenhydramine (5 x 10^{-6} g/ml) on the maximum rate of rise of the action potential are shown in Figure 20. The same experimental protocol as used for chlorpromazine was used in this case. Figure 21 illustrates the results of five such experiments done

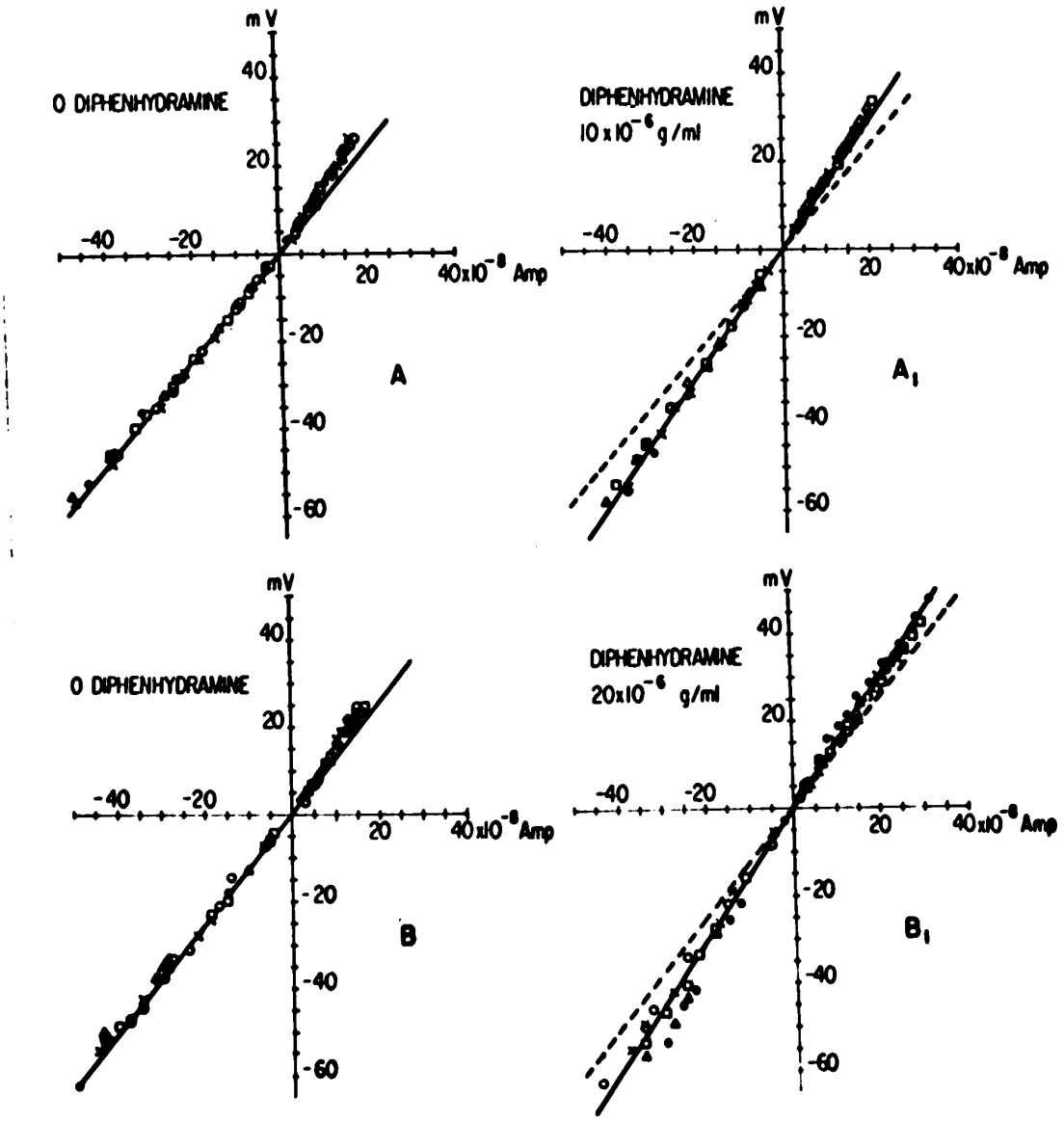

Figure 19. Effects of diphenhydramine treatment on the current-voltage relation of frog sartorius muscle fibres. The different symbols on each graph represent individual muscle fibres. A and ß are the control responses obtained from two separate muscles in Ringer's solution. A_1 and B_1 are the responses recorded after 2 hours exposure to diphenhydramine. The solid linear regression lines were drawn to fit the data obtained at the specified concentration. The broken lines represent the control linear regression line (diphenhydramine = 0) superimposed to show the effects of diphenhydramine treatment. The potentials were measured at the end of 2 msec current pulses of the specified intensity.

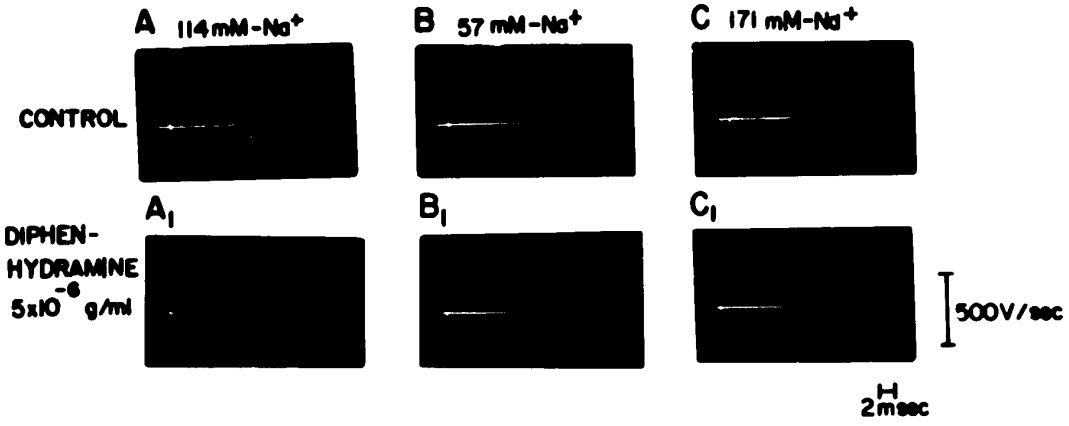

Figure 20. Differentiated, intracellularly recorded action potentials showing the effect of diphenhydramine on the maximum rate of rise of the action potential. The fibres were stimulated with an extracellular pore electrode at a point distant from the recording electrode. The stimulus artifact appears at the start of each record.

The peak of each record represents the maximum rate of rise of the action potential under the specified condition. Responses were obtained from different fibres of the same muscle. Sodium concentration of the bathing solution is shown above the records. The control records with low and high sodium concentration were obtained after 30 minutes soaking in either case. The muscle was exposed to normal Ringer's solution containing diphenhydramine up to 2 hours, and for at least 30 minutes in low and high sodium Ringer's with diphenhydramine before each recording. A, B, and C, without diphenhydramine; A_1, B_1, and C_1, 5×10^{-6} g/ml diphenhydramine.

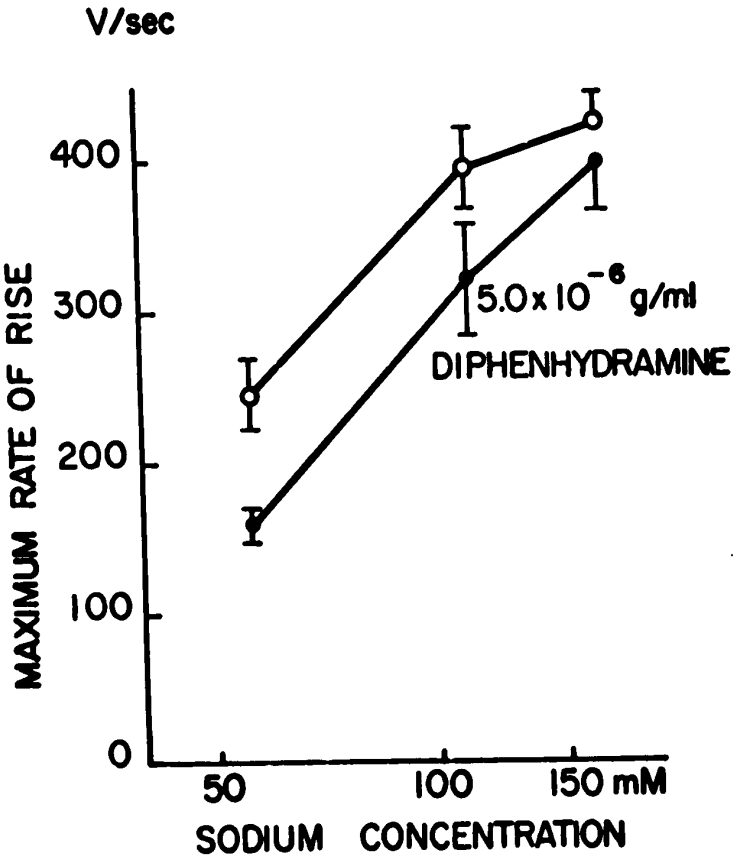

Figure 21. Effect of extracellular sodium concentration and of diphenhydramine on the maximum rate of rise of intracellularly recorded action potentials. Upper curve, Ringer's solution without diphenhydramine; lower curve, with diphenhydramine (5×10^{-6} g/ml). Mean and standard error derived from the mean values obtained in each of five separate preparations. Ordinate, maximum rate of rise of the action potential in V/sec; abscissa, sodium concentration of the bathing medium in mM.

with diphenhydramine and various external Na^+ concentrations. They suggest a competition between the sodium ions and diphenhydramine. The decrease in the maximum rate of rise by diphenhydramine was quickly antagonized by raising Na^+ in the bathing medium. In addition, it was found that increasing the extracellular sodium concentration readily overcame the diphenhydramine-induced reduction of the overshoot of the action potential (Figure 22). The observations that an augmentation of the extracellular sodium concentration counteracts the depressant actions of diphenhydramine on the rate of rise and the overshoot of the action potential lend further support to the mechanism of action for diphenhydramine proposed here and proposed previously by Weidmann (1955).

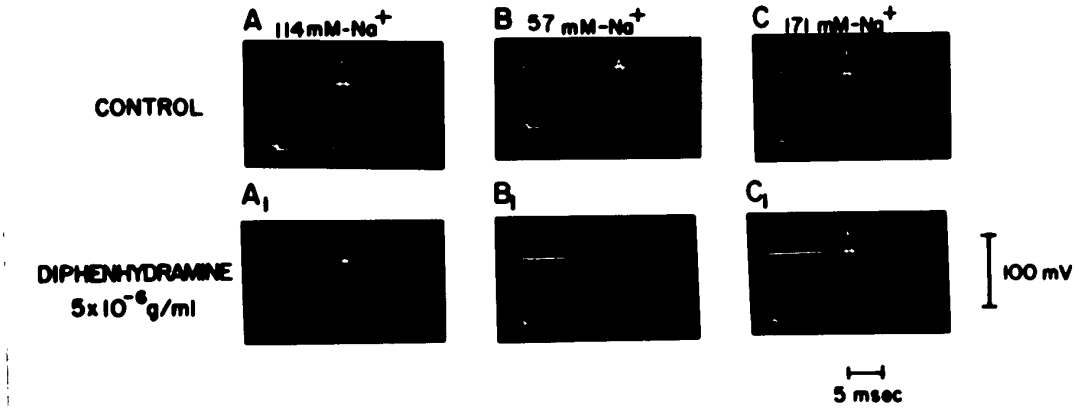

Figure 22. Effect of diphenhydramine on the overshoot of the intracellularly recorded action potentials. Sodium concentration of the bathing medium is indicated above the records. The stimulus artifact appears at the start of each action potential. The horizontal line across the upper part of all the records indicates zero potential.

Action potentials were recorded from different fibres of the same muscle. The control records with low and high sodium were taken after 30 minutes soaking in either case. The muscle was exposed to normal Ringer's solution containing diphenhydramine for 2 hours, and for 30 minutes in low and high sodium Ringer's before each recording. A, B, and C, without diphenhydramine; A_1, B_1, and C_1, 5×10^{-6} g/ml diphenhydramine. In this muscle the mean overshoot potential (mV $\pm$ S.E.) under the various conditions was: A, 29.8 $\pm$ 2.7 (10 fibres); B, 14.6 $\pm$ 1.6 (8 fibres); C, 36.0 $\pm$ 2.4 (11 fibres); A_1, 7.5 $\pm$ 2.8 (9 fibres); B_1, 1.5 $\pm$ 2.2 (6 fibres); and C_1, 23.8 $\pm$ 1.7 (12 fibres).

(F) SCOPOLAMINE (HYOSCINE):

i. Studies with Extracellular Stimulation. Treatment of the muscle bundles with scopolamine diminished the excitability and reduced the amplitude of the compound action potentials in a reversible manner. The details of the procedure used for stimulation and recording can be found on page 54. The results of all experiments with this technique are summarized in Figure 23. Application of scopolamine in a range of 0.25 to 0.5 x 10^{-4} g/ml did not produce any apparent change both in the excitability and in the amplitude of the action potential. However, higher concentrations progressively depressed the excitability, and reduced the action potential height. A complete but reversible conduction block occurred after a lapse of 30 minutes when the muscles were exposed to 25 x 10^{-4} g/ml scopolamine. Restoration of responses from the highest concentration took place after about 60 minutes washing in Ringer's solution.

The addition of 25 x 10^{-4} g/ml scopolamine hydrobromide reduced the pH of normal Ringer's solution from 7.6 to about 7.2. No correction of the pH was attempted, since the addition of alkali ($NaHCO_3$ or NaOH) would indirectly increase the sodium content of the Ringer's solution, and the latter would in turn affect the properties under investigation. Moreover, it was found that decreasing the pH of the normal Ringer's solution to between 7.2 to 7.3 produced no significant alteration in the amplitude of the compound action potential and in the excitability when compared with the control responses obtained in normal Ringer's fluid (pH 7.6).

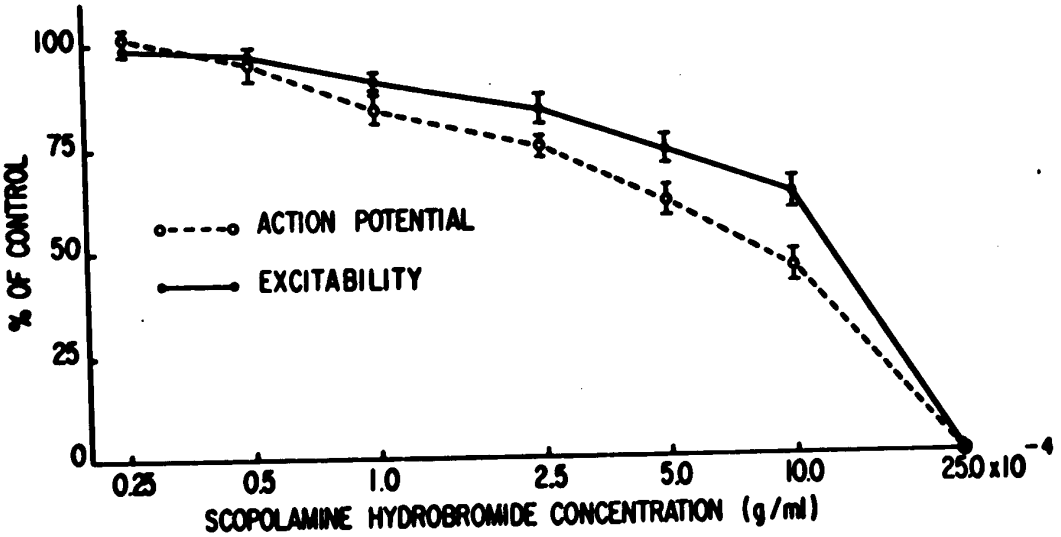

Figure 23. Effects of various concentrations of scopolamine on excitability, calculated as the inverse of the threshold current, and on the maximum size of the compound action potential of frog sartorius muscle strips. Each mean and standard error was calculated from the mean responses of each of six preparations.

The osmolarity of the Ringer's solution was slightly increased (from 234 to 246 milliosmols) after adding 25 x 10^{-4} g/ml of scopolamine hydrobromide. It will be shown later that such a trivial increase in the osmolarity is rather inconsequential when comparisons are made with those experiments performed under identical conditions with iso-osmolar concentration of sucrose (p. 160).

ii. Studies with Intracellular Electrodes. Figure 24 shows superimposed records of the electrotonic potentials, the action potentials and the amounts of current required to produce these depolarizations in the fibre membrane with and without scopolamine. Some of the values obtained in experiments of this type are presented in Table IV. Inspection of Figure 24 and Table IV indicates that both the threshold depolarization and the threshold current are increased in the presence of scopolamine. The critical level of the membrane potential (firing level) and the overshoot potential are decreased by exposing the muscle fibres to solutions containing scopolamine. Often there was no overshoot with 5 x 10^{-4} g/ml scopolamine (Figure 24-C_1), and in few fibres the peak of the action potential was actually below the zero potential base line. The resting membrane potentials remain unaltered in the scopolamine treated muscles (Table IV). The increase in the threshold current accompanied by an increase in the threshold depolarization, and the decrease in the overshoot potential indicate that the underlying mechanism of action for scopolamine seems to be the depression of the sodium conductivity, the latter being so vital for the genera-

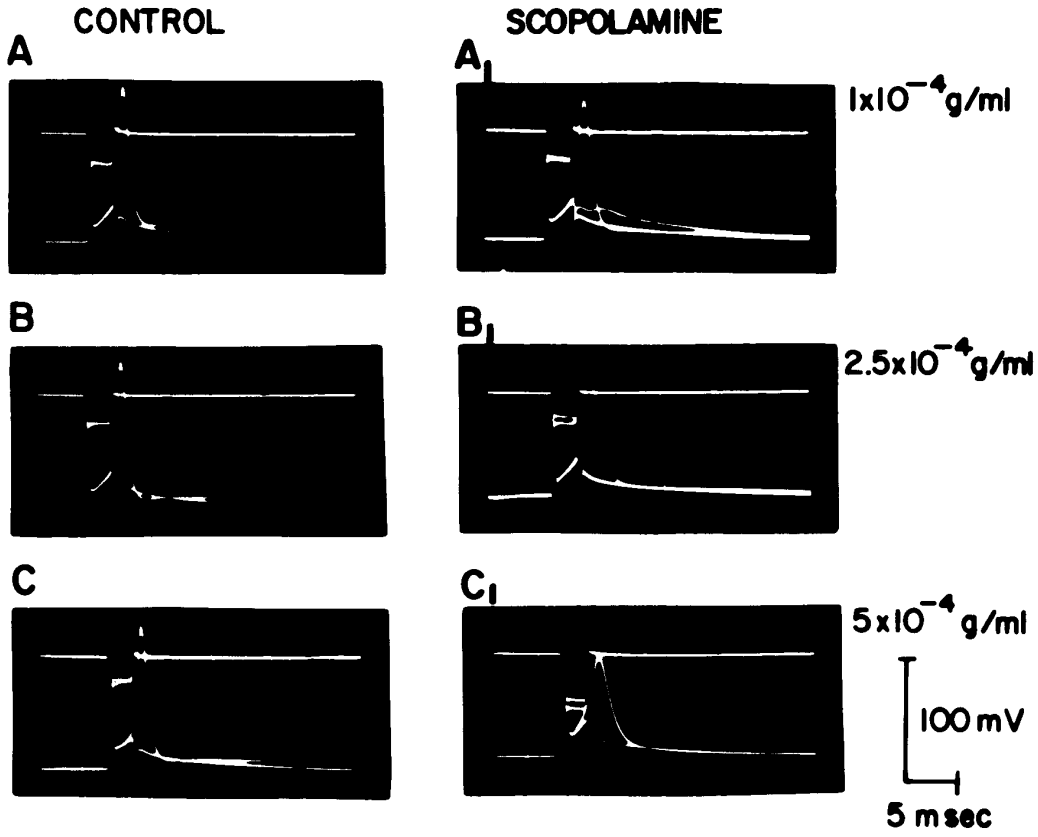

Figure 24. Effect of scopolamine on the intracellularly recorded action potentials from the frog sartorius muscle fibres. A, B, and C, are the control responses from separate fibres of the same muscle. A_1, B_1, and C_1, are the recordings done after 30 minutes exposure to a specified concentration of scopolamine. Upper traces show stimulus current records, lower traces indicate the passive electrotonic depolarizations and the action potentials produced by 2 msec current pulses.

TABLE IV

Effects of scopolamine on some membrane electrical properties of frog sartorius muscle fibres. The muscles were exposed to various concentrations of scopolamine for 30 minutes before the measurements were obtained.

Muscles (No.)	Fibres (No.)	Scopolamine Concentration (X 10^{-4} g/ml)	Resting Potential* (mV)	Threshold Depolarization (mV)	Threshold Current (X 10^{-7} Amp.)	Effective Resistance (K.Ω)	τ_m (msec)
4	42	0	86.8 ± 1.6	26.1 ± 1.3	1.7 ± 0.14	255 ± 8	12.7 ± 0.5
4	26	1.0	87.6 ± 1.2	29.8 ± 0.8	1.9 ± 0.05	262 ± 10	14.5 ± 1.4
3	21	2.5	89.0 ± 2.4	36.3 ± 1.6**	2.1 ± 0.12	285 ± 13**	16.8 ± 1.2**
3	17	5.0	86.6 ± 2.3	42.2 ± 1.8**	2.8 ± 0.21**	345 ± 9**	18.2 ± 1.5**

* Mean ± S.E., derived from mean values for each muscle.

** Treatment means are significantly different from the control means ($P < 0.05$).

tion of action potential in the skeletal muscle membrane.

iii. Effect on the Current-Voltage Relation. Figure 25 reveals that scopolamine produces a counterclockwise shift of the current-voltage curves. The magnitude of this shift is approximately the same with 2.5 and 5 x 10^{-4} scopolamine. This counterclockwise rotation of the curves is most likely the manifestation of the increased effective membrane resistance. The increase observed in the τ_m, and in the effective membrane resistance under the influence of scopolamine (Table IV) also conform with this suggestion. From these observations it may be inferred that the passive membrane conductance is also altered by scopolamine treatment. The other change produced by scopolamine is the suppression of the local potential (Figure 25), suggesting a specific decrease of sodium conductivity in the fibre membrane.

iv. Effect on the Maximum Rate of Rise of the Action Potential. The action of scopolamine on the differentiated intracellularly recorded action potentials in normal, low and high sodium Ringer's is shown in Figure 26. The soaking interval was 30 minutes in each case. Graph in Figure 27 shows the results of four such experiments. These results suggest a competitive inhibition, with the effect of 2.5 x 10^{-4} g/ml scopolamine being approximately equivalent to that of a 50% reduction in the external sodium concentration. It can be noted from Figure 26-C_1, that the decrease in the maximum rate of rise of the action potential in the presence of scopolamine can be

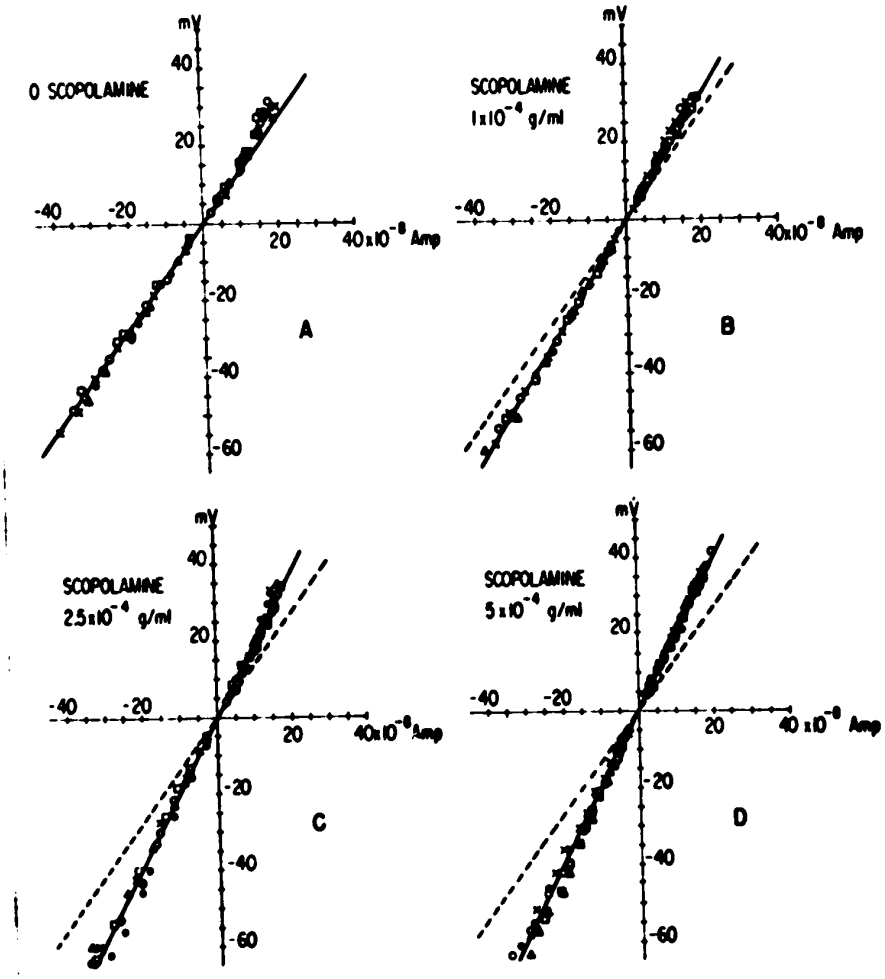

Figure 25. Effects of scopolamine treatment on the current-voltage relation of frog sartorius muscle fibres. The different symbols on each graph represent individual muscle fibres. A, represents the control responses obtained from separate fibres of the same muscle in Ringer's solution; B, C, and D, are the responses recorded after 30 minutes exposure to scopolamine. The solid linear regression lines were drawn to fit the data obtained at the specified concentration. The broken lines represent the control linear regression line (scopolamine = 0) superimposed to show the effects of scopolamine treatment. The potentials were measured at the end of 2 msec current pulses of the specified intensity.

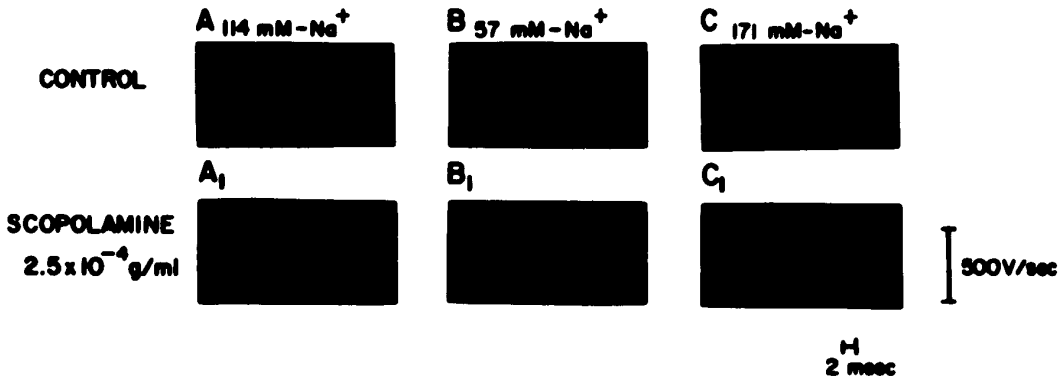

Figure 26. Differentiated, intracellularly recorded action potentials showing the effect of scopolamine on the maximum rate of rise of the action potential. The fibres were stimulated with an extracellular pore electrode at a point distant from the recording electrode. The stimulus artifact appears at the start of each record.

The peak of each record represents the maximum rate of rise of the action potential under the specified condition. Responses were obtained from separate fibres of the same muscle. Sodium concentration of the bathing solution is shown above the records. The muscle was exposed to altered sodium and scopolamine concentrations for at least 30 minutes before each recording. A, B, and C, without scopolamine; A_1, B_1, and C_1, 2.5×10^{-4} g/ml scopolamine.

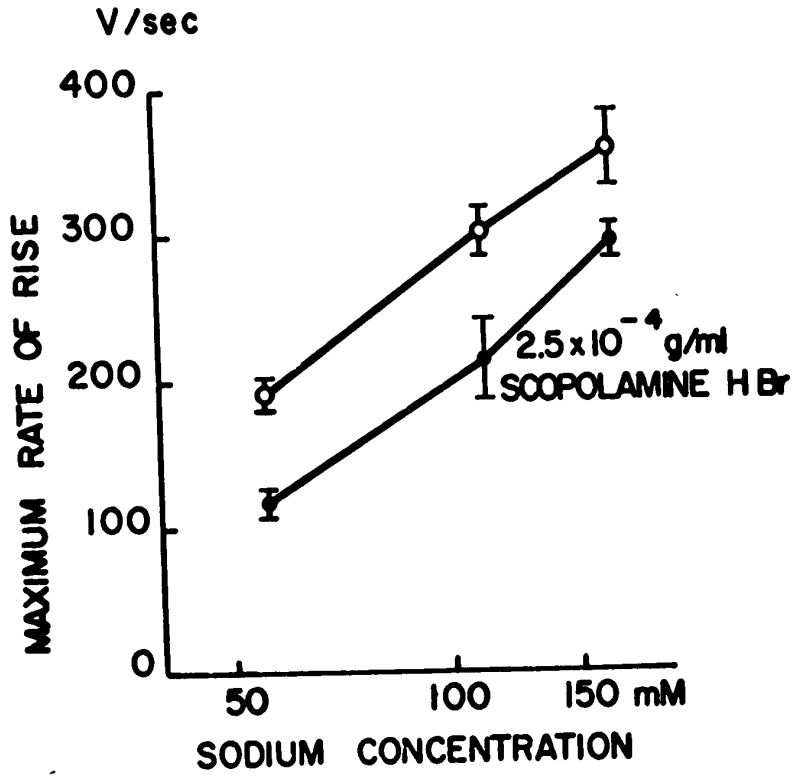

Figure 27. Effect of extracellular sodium concentration and of scopolamine on the maximum rate of rise of intracellularly recorded action potentials. Upper curve, Ringer's solution without scopolamine; lower curve, with scopolamine (2.5×10^{-4} g/ml). Mean and standard error derived from the mean values obtained in each of four separate preparations. Ordinate, maximum rate of rise of the action potential in V/sec; abscissa, sodium concentration of the bathing medium in mM.

overcome by increasing the sodium concentration in the extracellular medium. Similarly the scopolamine-induced depression of the overshoot potential can be restored by adding excess sodium to the Ringer's solution (Figure 28-C_1).

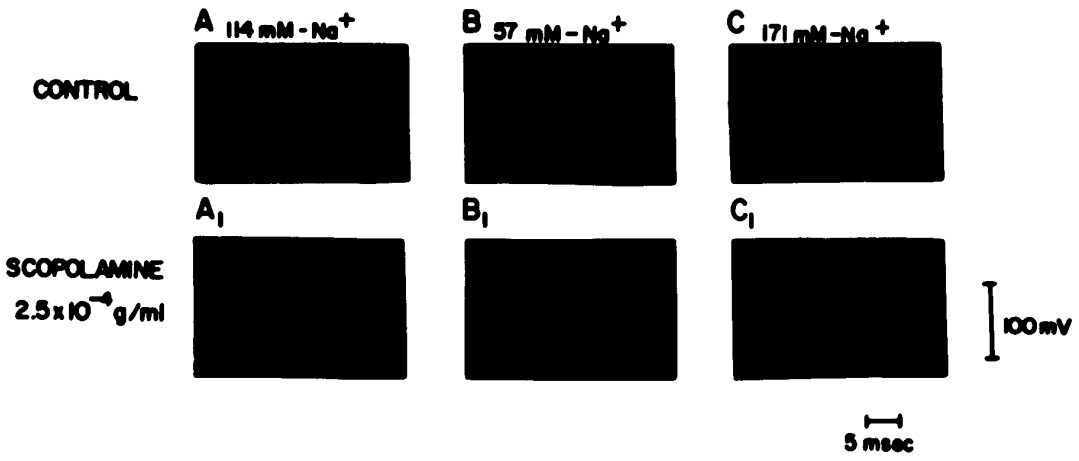

Figure 28. Effect of scopolamine on the overshoot of the intracellularly recorded action potentials. Sodium concentration of the bathing medium is indicated above the records. The stimulus artifact appears at the start of each action potential. The horizontal line across the upper part of all the records indicates zero potential.

Action potentials were recorded from separate fibres of the same muscle. The muscle was exposed to altered sodium and scopolamine concentrations for at least 30 minutes before each recording. A, B, and C, without scopolamine; A_1, B_1, and C_1, 2.5 x 10^{-4} g/ml scopolamine. In this muscle the mean overshoot potential (mV $\pm$ S.E.) under the various conditions was: A, 26.7 $\pm$ 1.8 (25 fibres); B, 8.9 $\pm$ 1.6 (18 fibres); C, 35.2 $\pm$ 2.1 (12 fibres); A_1, 12.5 $\pm$ 1.6 (14 fibres); B_1, 0.5 $\pm$ 1.4 (8 fibres); and C_1, 26.2 $\pm$ 1.2 (19 fibres).

(G) MEPERIDINE (DEMEROL):

i. Studies with Extracellular Stimulation. The results obtained from five muscle strips treated with various amounts of meperidine-Ringer's solution are shown in Figure 29. Addition of 0.25 and 0.5 x 10^{-5} g/ml meperidine into Ringer's solution was almost without any effect on the compound action potential amplitude and on the excitability of the frog sartorius muscle fibres. However, transferring the muscle for 30 minutes into Ringer's containing 10 x 10^{-5} g/ml meperidine the height of the action potential was reduced to 19.8 $\pm$ 4.7%, and at the same time the excitability was depressed to about 64.9 $\pm$ 6.6% as compared with the control responses. The recovery of the fibres from the highest concentrations of meperidine took place after about 60 to 90 minutes following a 30 minutes exposure.

ii. Studies with Intracellular Electrodes. Figure 30 shows the superimposed records of the subthreshold and threshold current pulses passed through one electrode and the resulting displacement of membrane potentials measured with the other. Measurements were taken from several fibres of different muscles with and without the presence of meperidine hydrochloride. The quantitative data thus obtained are summarized in Table V. Meperidine increased both the threshold depolarization of the fibre membrane and the amount of threshold current needed to generate action potentials. However, these effects of meperidine on the membrane excitability were not accompanied by any marked changes in the resting membrane potential (Table V). The size of the overshoot

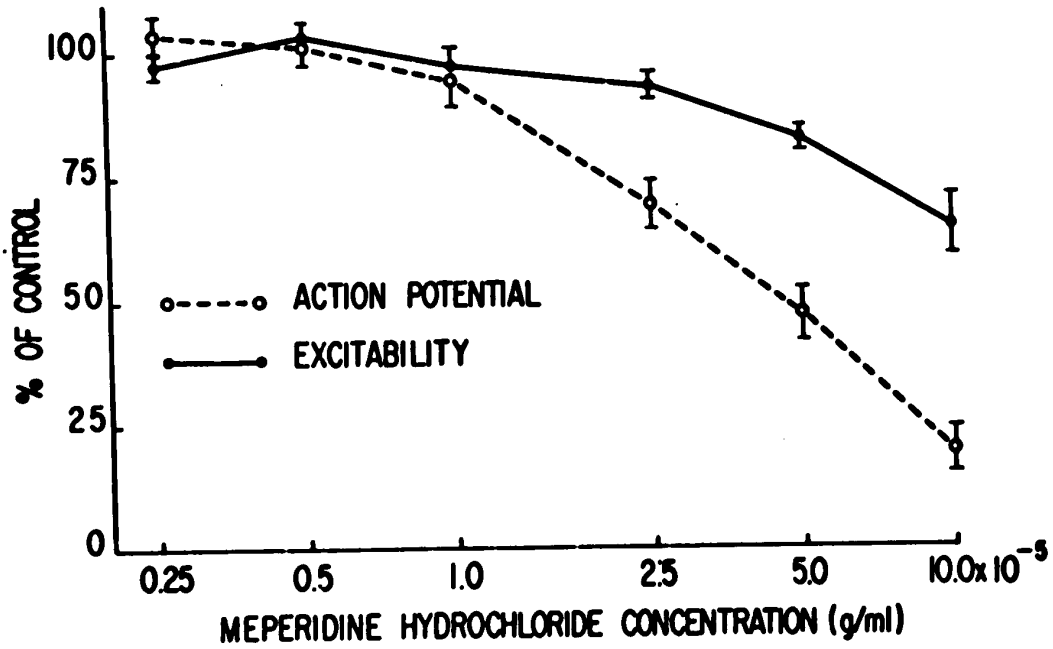

Figure 29. Effects of various concentrations of meperidine on the excitability, calculated as the inverse of the threshold current, and on the maximum size of the compound action potential of frog sartorius muscle strips. Each mean and standard error was calculated from the mean responses of each of five or six preparations.

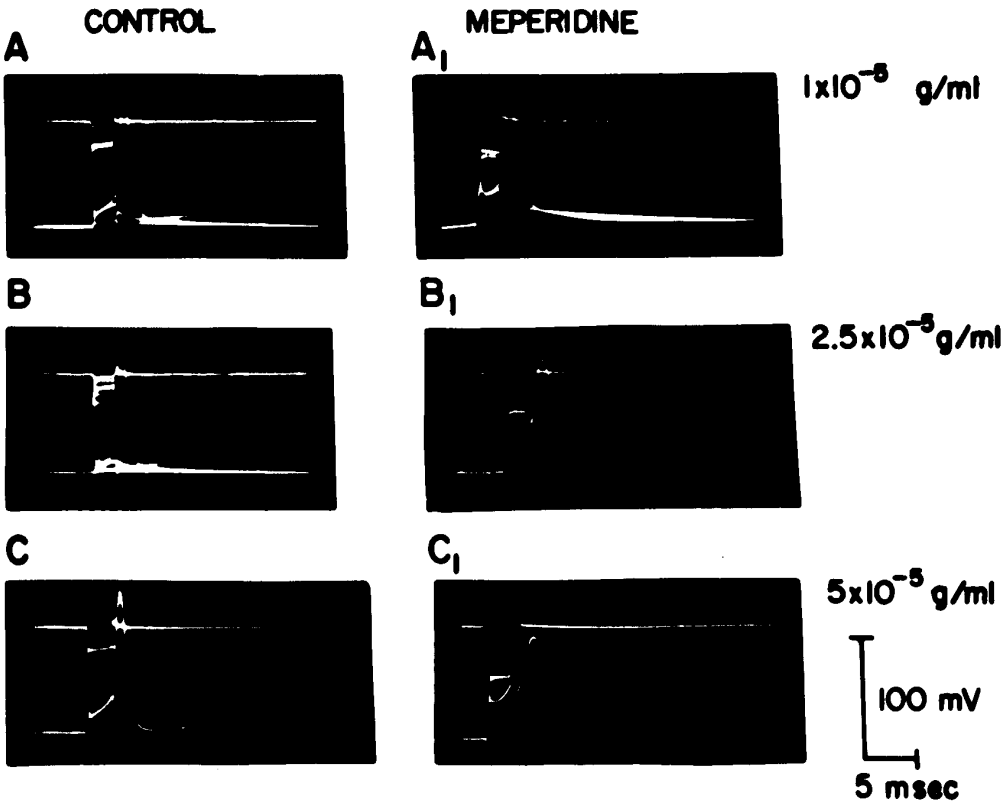

Figure 30. Effect of meperidine on the intracellularly recorded action potentials from the frog sartorius muscle fibres. A, B, and C, are the control responses from separate fibres of the same muscle. A_1, B_1, and C_1, are the recordings done after 30 minutes exposure to a specified concentration of meperidine. Upper traces show stimulus current records, lower traces represent the passive electrotonic depolarizations and the action potentials produced by 2 msec current pulses.

TABLE V

Effects of meperidine on some membrane electrical properties of frog sartorius muscle fibres. The muscles were treated with various concentrations of meperidine for 30 minutes before the measurements were obtained.

Muscles (No.)	Fibres (No.)	Meperidine Concentration (X 10^{-5} g/ml)	Resting Potential* (mV)	Threshold Depolarization (mV)	Threshold Current (X 10^{-7} Amp.)	Effective Resistance (K.Ω)	τ_m (msec)
4	35	0	87.3 ± 1.7	27.4 ± 1.8	1.7 ± 0.07	264 ± 11	12.5 ± 1.4
4	24	1.0	93.1 ± 2.8	32.2 ± 1.6**	1.9 ± 0.12	258 ± 13	13.2 ± 1.3
3	17	2.5	88.3 ± 1.5	35.2 ± 1.9**	2.7 ± 0.14**	266 ± 12	13.8 ± 1.7
3	15	5.0	86.9 ± 1.8	41.5 ± 1.1**	3.1 ± 0.16**	271 ± 10	14.2 ± 0.9

* Mean ± S.E., derived from the mean values for each muscle.

** Treatment means are significantly different from the control means ($P < 0.05$).

potential is either reduced (Figure 30-A_1 and B_1), or completely eliminated (Figure 30-C_1) after the meperidine treatment. In majority of the fibres studied the peak of the action potential was below the zero potential base line when the muscles were immersed in solutions containing 5×10^{-5} meperidine. In addition, sporadic blockade of the action potentials was observed when 5×10^{-5} g/ml meperidine was applied to the fibres.

Records A_1, B_1, and C_1 in Figure 30 show that following the meperidine treatment there is an apparent prolongation in the action potential duration as compared with the corresponding controls. However, no quantitative measurements for the prolongation of the duration of the action potentials were attempted. These results suggest that apart from exerting its depressant actions on the sodium conductance, meperidine also tends to inhibit the increase in the membrane potassium conductivity which is normally associated with the falling phase of the skeletal muscle action potential.

Comparison of the data in Table V would indicate that the effective membrane resistance, and the membrane time constant (τ_m) are slightly increased by applying meperidine to the frog sartorius muscle. These observations indicate that the input resistance (effective resistance) of the membrane at the point of stimulation stays practically unchanged after meperidine treatment.

iii. Effect on the Current-Voltage Relation. The effects of various concentrations of meperidine on the current-voltage relation

of the frog sartorius muscle fibres were determined in 3 to 4 muscles, and the results of a typical study are shown in Figure 31. The same linear regression line passing through the origin is drawn on all four graphs. It can be seen that meperidine does not alter the slope of this linear relation, which means that the passive membrane conductance remains unaltered. The records in Figure 31 also show that the predominant change brought about by meperidine (25 and 50 x 10^{-6} g/ml) is the suppression of the local response (Figure 31-C and D), because all the points following the meperidine treatment fit closely on the linear regression line.

iv. Effect on the Maximum Rate of Rise of the Action Potential. Typical differentiated action potentials recorded from single muscle fibres in normal Ringer's solution, and in Ringer's solution containing 0.5 times and 1.5 times sodium than the usual Ringer's are presented in Figure 32. As can be seen, treating the muscle with meperidine has an effect similar to that of reducing the extracellular sodium concentration. The results of four such experiments in which the maximum rate of rise was determined in altered sodium and meperidine concentrations are shown graphically in Figure 33. They suggest a competitive inhibition, with the effect of 2.5 x 10^{-5} g/ml meperidine being approximately equivalent to a 50% reduction in the extracellular sodium concentration. As mentioned earlier, exposure of the fibres to meperidine-Ringer's solution produces a diminution of the overshoot potential, the latter effect can largely

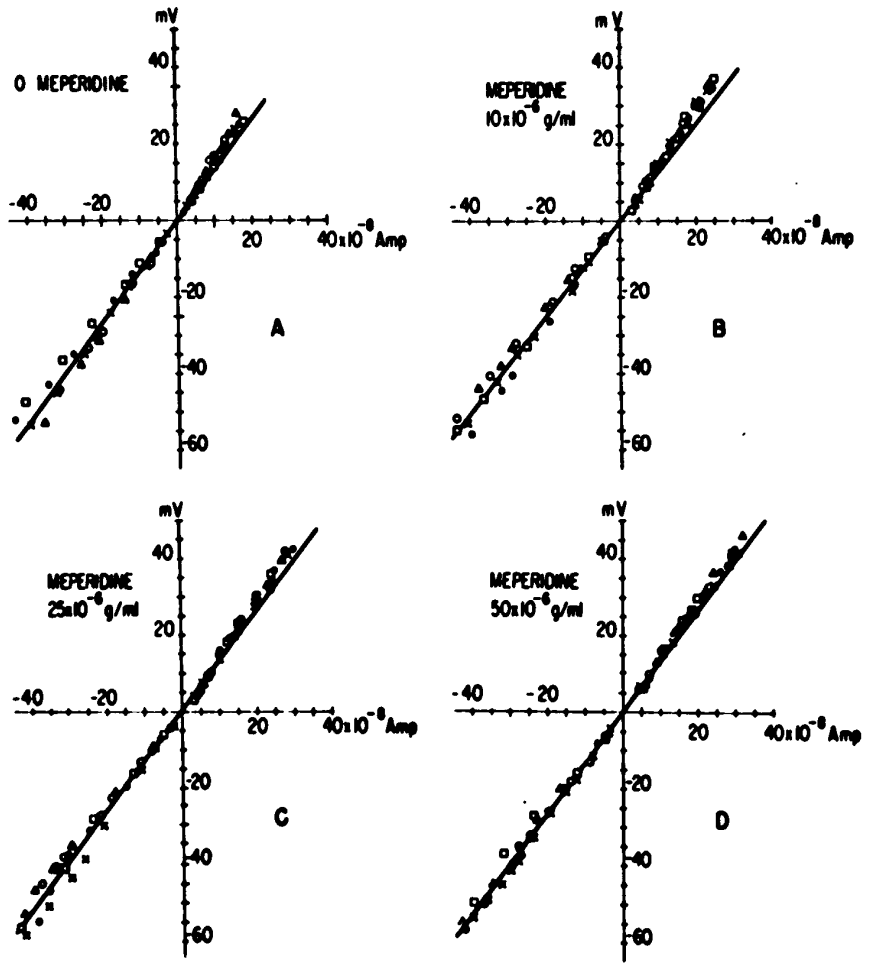

Figure 31. Effects of meperidine treatment on the current-voltage relation of frog sartorius muscle fibres. The different symbols on each graph represent individual muscle fibres. A, represents the control responses obtained from different fibres of the same muscle in Ringer's solution. B, C, and D, are the responses recorded after 30 minutes exposure to meperidine. The same linear regression line is drawn on all four graphs. The potentials were measured at the end of 2 msec current pulses of the specified intensity.

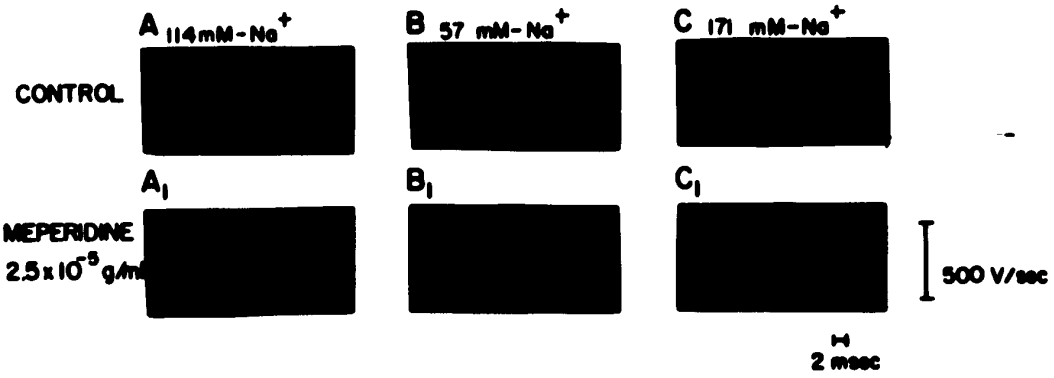

Figure 32. Differentiated, intracellularly recorded action potentials showing the effect of meperidine on the rate of rise of the action potential. The fibres were stimulated with an extracellular pore electrode at a point distant from the recording electrode. The stimulus artifact appears at the start of each record.

The peak of each record indicates the maximum rate of rise of the action potential under the specified concentration. Sodium concentration of the bathing medium is shown above the records. The muscle was exposed to altered sodium and meperidine concentrations for 30 minutes before each recording. A, B, and C, without meperidine; A_1, B_1, and C_1, 2.5×10^{-5} g/ml meperidine.

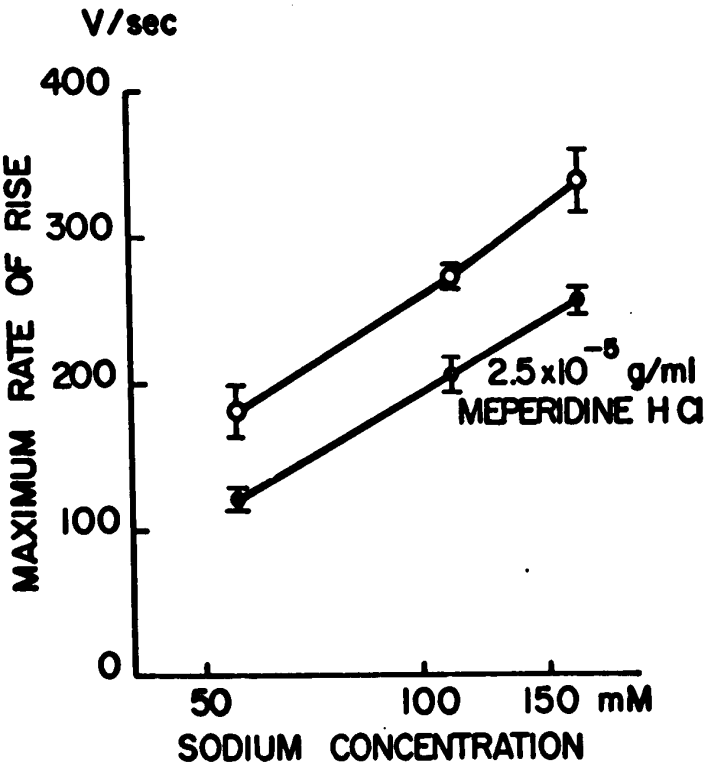

Figure 33. Effect of extracellular sodium concentration and of meperidine on the maximum rate of rise of intracellularly recorded action potentials. Upper curve, Ringer's solution without meperidine; lower curve, with meperidine (2.5×10^{-5} g/ml). Mean and standard error derived from the mean values obtained in each of four separate preparations. Ordinate, maximum rate of rise of the action potential in V/sec; abscissa, sodium concentration of the Ringer's solution in mM.

be antagonized by increasing the sodium concentration into the bathing fluid (Figure 34).

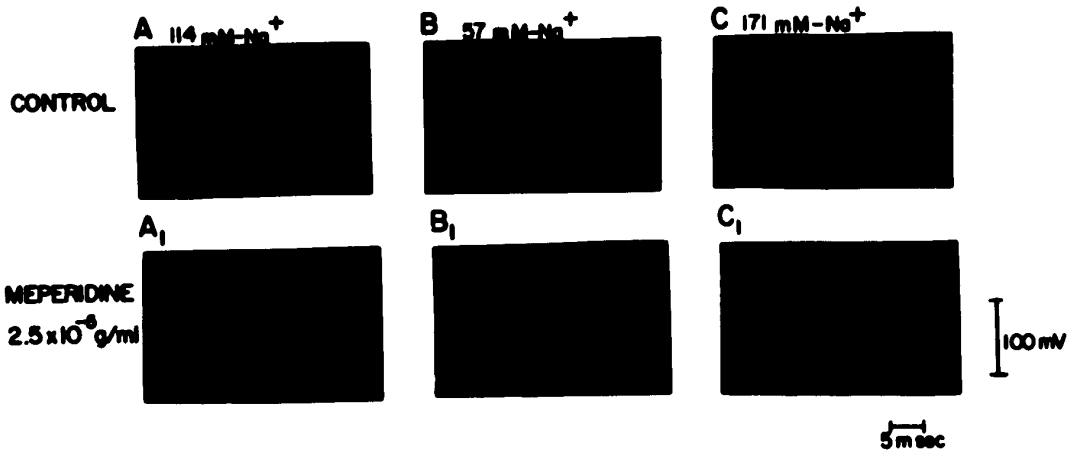

Figure 34. Effect of meperidine on the overshoot of the intracellularly recorded action potentials. Sodium concentration of the Ringer's solution is indicated above the records. The stimulus artifact appears at the start of each action potential. The horizontal line across the upper part of all the records indicates zero potential base line.

Action potentials were recorded from different fibres of the same muscle. The muscle was exposed to altered sodium and meperidine concentrations for a period of 30 minutes before each recording. A, B, and C, without meperidine; A_1, B_1, and C_1, 2.5×10^{-5} g/ml meperidine. In this muscle the mean overshoot potential (mV $\pm$ S.E.) under the various conditions was: A, 28.2 $\pm$ 1.4 (20 fibres); B, 10.9 $\pm$ 2.2 (15 fibres); C, 34.2 $\pm$ 2.6 (19 fibres); A_1, 14.3 $\pm$ 1.7 (20 fibres); B_1, 1.5 $\pm$ 1.9 (13 fibres); and C_1, 19.6 $\pm$ 1.3 (9 fibres)

(H) MORPHINE:

i. Studies with Extracellular Stimulation. The effects of morphine were studied on the isolated sartorius muscles of the frog. Application of morphine to the muscle bundles in concentrations of 0.25 to 1.0 x 10^{-4} g/ml hardly affected either the amplitude of the compound action potential or the excitability of the fibres (Figure 35). On the other hand, soaking the muscles into 25 x 10^{-4} g/ml of morphine for 30 minutes markedly reduced the size of the compound action potential (15.4 $\pm$ 2.5%), and depressed the excitability to 62 $\pm$ 3.1% as compared with the control responses. The recovery from this effect took place in about 45 to 75 minutes after the removal of the drug.

Addition of 25 x 10^{-4} g/ml morphine sulphate into the normal Ringer's solution produced a slight decrease in the pH, viz., from about 7.5 to about 7.2. No correction of the pH was made, and the reasons for not making such a correction were outlined before. Although the osmolarity of the ordinary Ringer's solution was slightly increased by the presence of 25 x 10^{-4} g/ml morphine sulphate (from 233 to 240 milliosmols), this much change in the osmolarity is of minor importance as will be shown under the effects produced by iso-osmolar sucrose-Ringer's solution (p. 160).

ii. Studies with Intracellular Electrodes. The results of the experiments done with two microelectrodes into the same muscle fibre are listed in Table VI. Increasing concentrations of morphine

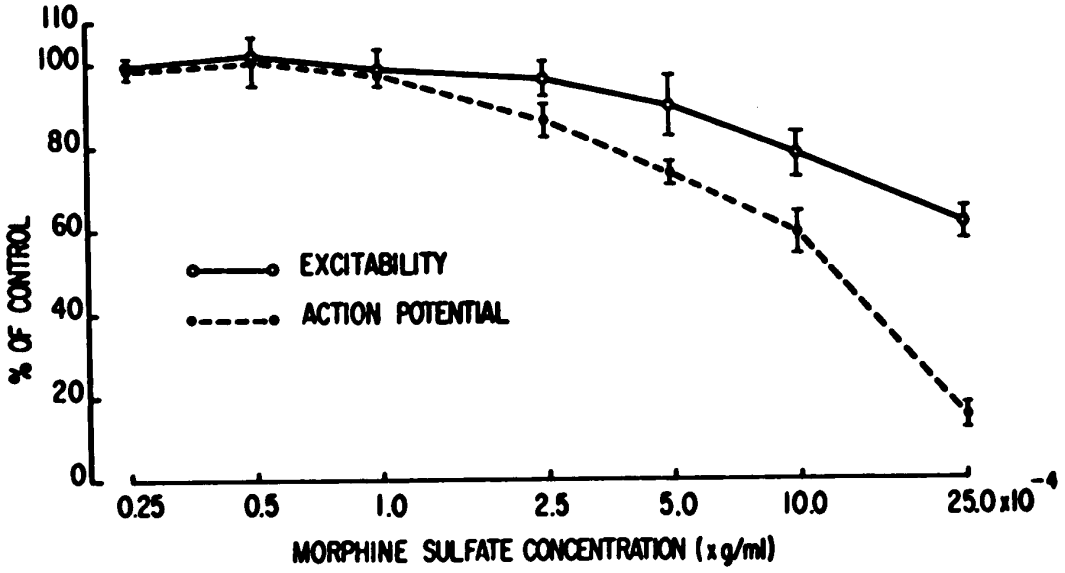

Figure 35. Effects of various concentrations of morphine on the excitability, calculated as the inverse of the threshold current, and on the maximum size of the compound action potential of frog sartorius muscle strips. Each mean and standard error was calculated from the mean responses of each of six preparations.

TABLE VI

Effects of morphine on some membrane electrical properties of frog sartorius muscle fibres. The muscles were treated with various concentrations of morphine for 30 minutes before the measurements were obtained.

Muscles (No.)	Fibres (No.)	Morphine Concentration (X 10^{-3} g/ml)	Resting Potential* (mV)	Threshold Depolarization (mV)	Threshold Current (X 10^{-7} Amp.)	Effective Resistance (K.Ω)	τ_m (msec)
4	32	0	90.5 ± 1.3	23.7 ± 1.6	1.5 ± 0.04	258 ± 8	12.6 ± 1.3
3	18	0.25	92.7 ± 1.4	25.4 ± 1.2	1.7 ± 0.06	253 ± 12	11.8 ± 1.5
3	14	0.5	91.2 ± 1.1	30.2 ± 0.9**	1.9 ± 0.08**	259 ± 6	13.8 ± 0.8
2	9	2.5	93.9 ± 1.6	36.9 ± 1.8**	2.4 ± 0.13**	263 ± 8	14.0 ± 1.1

* Mean ± S.E., derived from the mean values for each muscle.

** Treatment means are significantly different from the control means ($P < 0.05$).

produced an increase in both the threshold depolarization and the threshold current required to elicit a propagated action potential. As a consequence of the increased threshold depolarization, the firing level of the membrane was slightly decreased. The resting membrane potentials however remained essentially unaltered during the treatment with morphine (Table VI).

Superimposed records of the stimulating (depolarizing) subthreshold and threshold 2 msec current pulses and the simultaneously occurring changes in the membrane potential are illustrated in Figure 36. It can be observed that in addition to its effects on the threshold depolarization and on the threshold current, the actions of morphine are characterized by a decrease of the overshoot potential which was completely eliminated (in large number of fibres) by 2.5 x 10^{-3} g/ml of morphine (Figure 36-C_1). Quite often the action potentials failed to reach the zero potential base line, and occasionally the fibres were rendered totally inexcitable (did not fire action potential) when 2.5 x 10^{-3} g/ml morphine was added into the Ringer's solution. Like meperidine, treatment of the fibres with morphine considerably prolonged the duration of the action potential (Figure 36-B_1 and C_1). After soaking the muscle for 30 minutes into 2.5 x 10^{-3} g/ml morphine-Ringer's solution, the action potential appeared to acquire a "bell-shaped" configuration, that is, both the rate of rise and the rate of fall of the spike were markedly reduced by morphine, and at the same time the peak of the action potential became broad (Figure 36-C_1). In contrast, the most commonly observed action potentials in

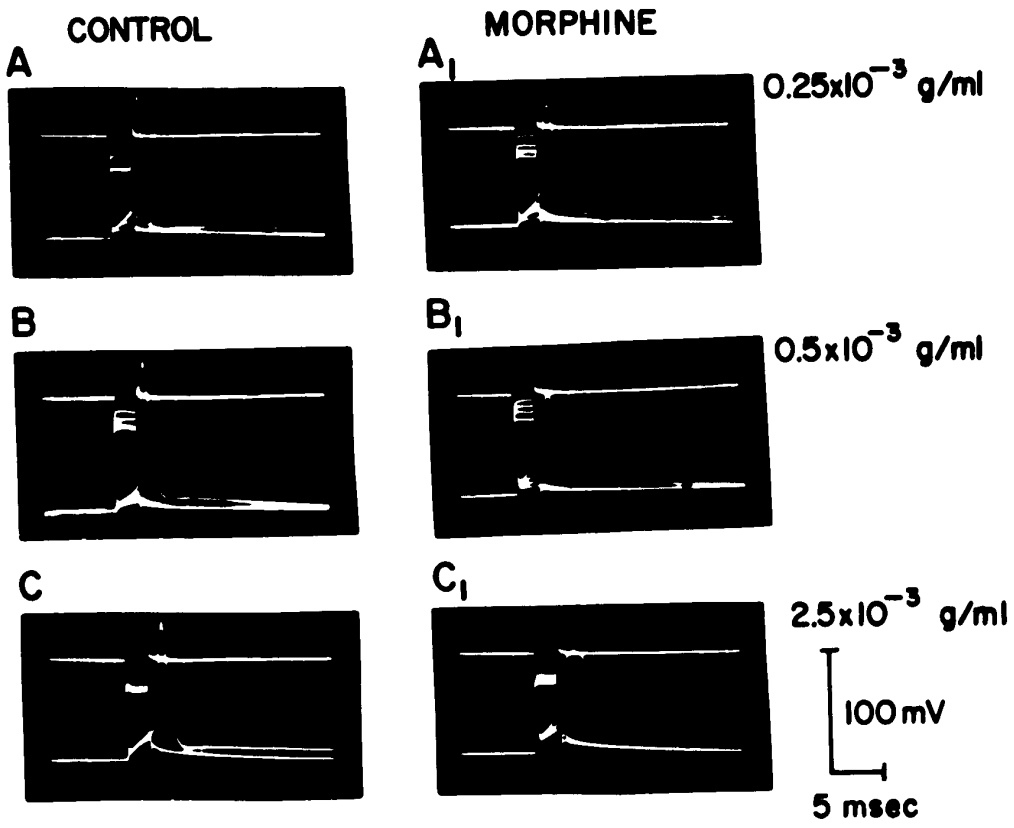

Figure 36. Effect of morphine on the intracellularly recorded action potentials from the frog sartorius muscle fibres. A, B, and C, are the control responses from separate fibres of the same muscle. A_1, B_1, and C_1, are the recordings done after 30 minutes exposure to a specified concentration of morphine. Upper traces show stimulus current records, while the lower traces represent the passive electrotonic depolarizations and the action potentials produced by 2 msec current pulses.

normal Ringer's solution had a sharp and cusped peak with a high initial rate of rise and a rapid rate of fall (Figure 36-A, B and C). These results are suggestive that morphine, like meperidine, suppresses both the sodium and potassium conductance in the active membrane of the frog sartorius muscle fibres.

iii. Effect on the Current-Voltage Relation. The actions of various concentrations of morphine on the current-voltage relation are presented in Figure 37. The same linear regression line passing through the origin is drawn on all the four graphs preceding and following the morphine application. Apparently, the slope of this linear relation does not change during the morphine treatment, from which it can be concluded that the membrane conductance in its passive state remains unaffected. However, the local response is slightly suppressed by morphine, since most of the points following morphine treatment fall on the linear regression line.

Inspection of the data in Table VI would indicate that there are no marked variations between the controls and the morphine treated muscles, so far as the membrane time constant (τ_m), and the effective membrane resistance are concerned. These results further support the suggestion already made that the passive membrane conductance remains virtually unaltered by morphine treatment.

iv. Effect on the Maximum Rate of Rise of the Action Potential. The effect of 2.5×10^{-4} g/ml morphine, along with various

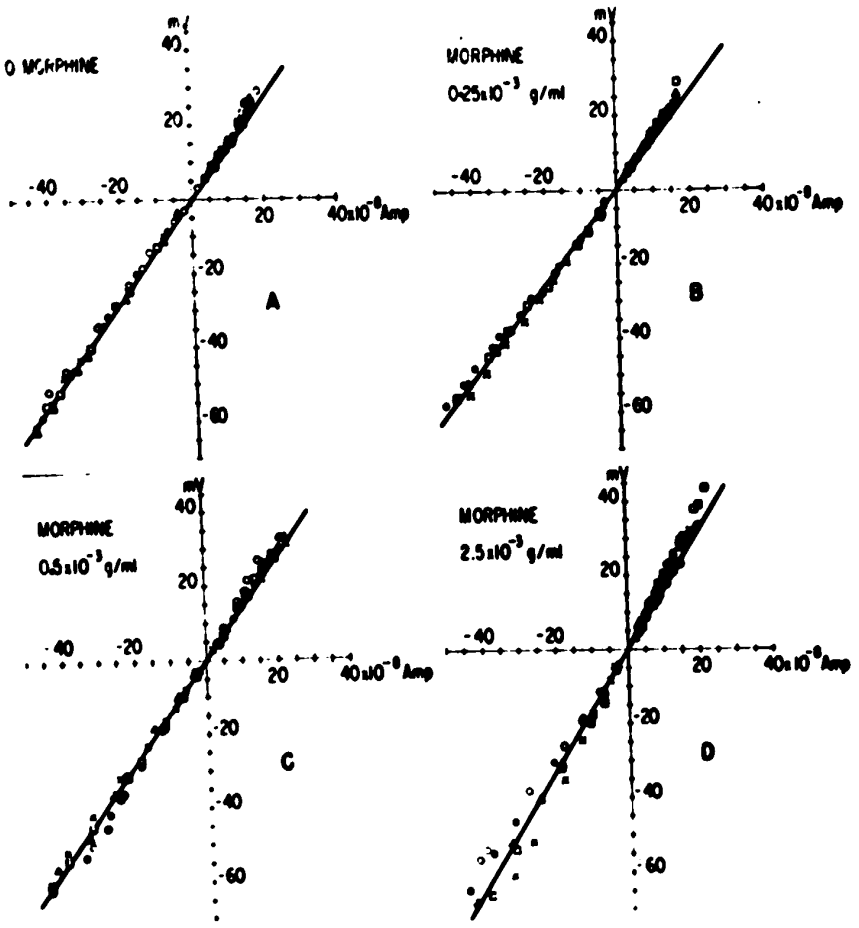

Figure 37. Effects of morphine treatment on the current-voltage relation of frog sartorius muscle fibres. The different symbols on each graph represent individual muscle fibres. A, represents the control responses obtained from different fibres of the same muscle in Ringer's solution. B, C, and D, are the responses recorded after 30 minutes exposure to morphine. The same linear regression line is drawn on all four graphs. The potentials were measured at the end of 2 msec current pulses of the specified intensity.

concentrations of Na^+ in Ringer's solution, on the maximum rate of rise of the action potential is illustrated in Figure 38. The data obtained in this fashion from the differentiated intracellularly recorded action potentials (four experiments) are plotted in Figure 39. It can be noted that the morphine-induced decrease of the maximum rate of rise of the action potential can be antagonized by increasing the Na^+ content of the bathing medium, as indicated by record C_1 in Figure 38 and also by Figure 39.

The other most noticeable change produced by morphine (2.5×10^{-4} g/ml) is the reduction, or the complete elimination of the overshoot potential (Figure 40-A_1 and B_1). Such an effect of morphine can likewise be overcome by the concomitant addition of sodium into Ringer's fluid (see record C_1 in Figure 40).

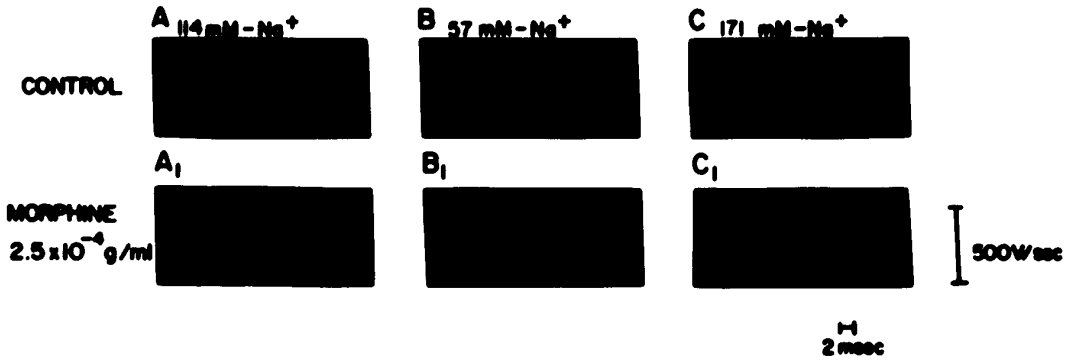

Figure 38. Differentiated, intracellularly recorded action potentials showing the effect of morphine on the rate of rise of the action potential. The fibres were stimulated with an extracellular pore electrode at a point distant from the recording electrode. The stimulus artifact appears at the start of each record.

The peak of each record indicates the maximum rate of rise of the action potential under the specified concentration. Sodium concentration of the bathing medium is shown above the records. The muscle was exposed to altered sodium and morphine concentrations for 30 minutes before each recording. A, B, and C, without morphine; A_1, B_1, and C_1, 2.5×10^{-4} g/ml morphine.

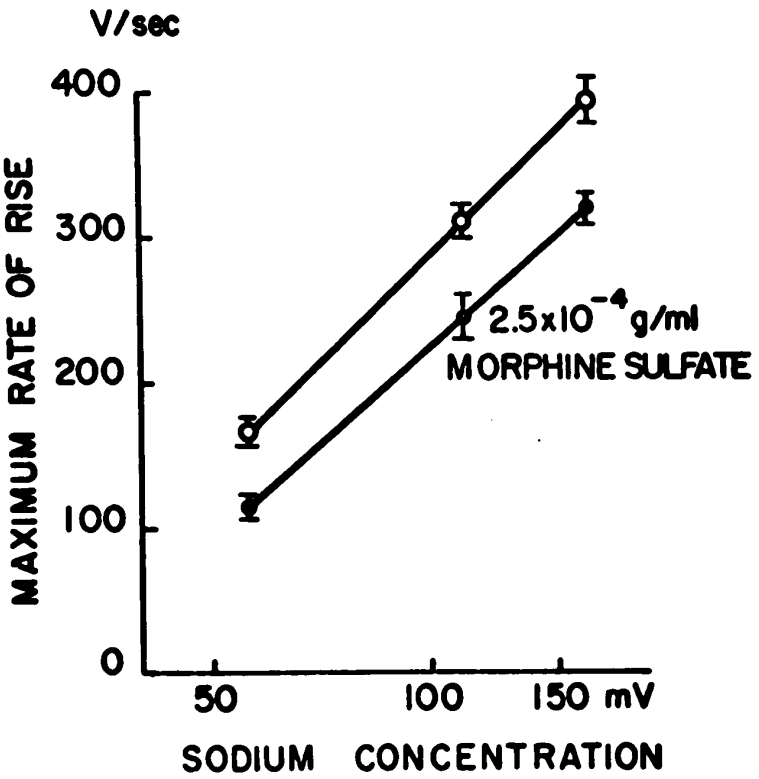

Figure 39. Effect of extracellular sodium concentration and of morphine on the maximum rate of rise of intracellularly recorded action potentials. Upper curve, Ringer's solution without morphine; lower curve, with morphine (2.5×10^{-4} g/ml). Mean and standard error derived from the mean values obtained in each of five separate preparations. Ordinate, maximum rate of rise of the action potential in V/sec; abscissa, sodium concentration of the Ringer's solution in mM.

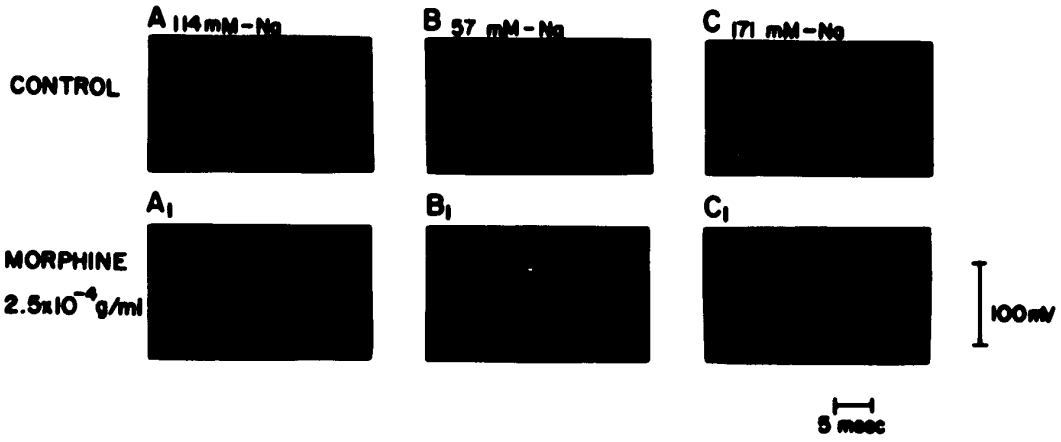

Figure 40. Effect of morphine on the overshoot of the intracellularly recorded action potentials. Sodium concentration of the Ringer's solution is indicated above the records. The stimulus artifact appears at the beginning of each action potential. The horizontal line across the upper part of all the records indicates zero potential base line.

Action potentials were recorded from different fibres of the same muscle. The muscle was exposed to altered sodium and morphine concentrations for 30 minutes before each recording. A, B, and C, without morphine; A_1, B_1, and C_1, 2.5×10^{-4} g/ml morphine. In this muscle the mean overshoot potential (mV $\pm$ S.E.) under the various conditions was: A, 25.1 $\pm$ 1.9 (24 fibres); B, 12.4 $\pm$ 2.4 (16 fibres); C, 30.1 $\pm$ 1.2 (19 fibres); A_1, 20.4 $\pm$ 2.3 (17 fibres); B_1, 1.4 $\pm$ 0.8 (13 fibres); and C_1, 28.5 $\pm$ 1.5 (18 fibres).

(I) GAMMA-HYDROXYBUTYRATE:

i. Studies with Extracellular Stimulation. When applied to the muscle strips in the range of 0.5 to 1.0 x 10^{-3} g/ml gamma-hydroxybutyrate (GHB) did not significantly reduce the amplitude of the compound action potential, and likewise the excitability of the fibres remained almost unaffected (Figure 41). However, after 30 minutes exposure to 15 x 10^{-3} g/ml GHB the excitability was decreased to about 77 $\pm$ 4.2% and the action potential size was reduced to roughly 66.1 $\pm$ 5% as compared with the control responses. The depression evoked by GHB in concentrations of 10 and 15 x 10^{-3} g/ml could be reversed in about 45 to 60 minutes; whereas with smaller concentrations the responses returned to the control level in about 30 minutes after the removal of the drug.

ii. Studies with Intracellular Electrodes. The results obtained with various concentrations of gamma-hydroxybutyrate are summarized in Table VII. It was found that the increase in the threshold depolarization was accompanied by a slight increase in the threshold current after the fibres had been treated with GHB. The resting membrane potential remained almost unchanged in the solutions containing GHB. From Figure 42 it can be noted that with increasing concentrations of GHB the amount of the depolarization required to initiate a propagated action potential (threshold depolarization) was increased, and the firing level of the membrane was slightly decreased. The overshoot of the action potential was nearly abolished when 10 x 10^{-3} g/ml

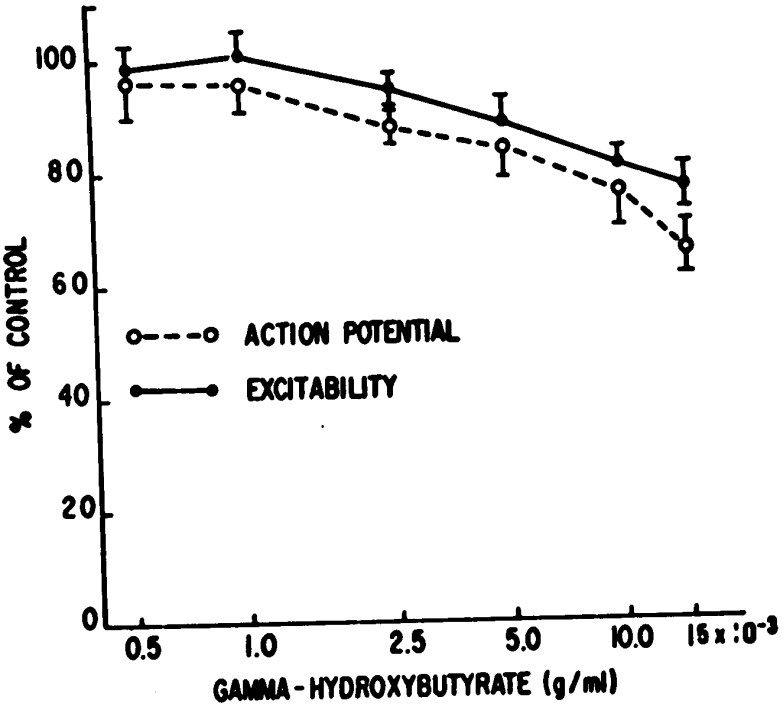

Figure 41. Effects of various concentrations of gamma-hydroxybutyrate on excitability, calculated as the inverse of the threshold, and on the maximum size of the compound action potential of frog sartorius muscle strips. Each mean and standard error was calculated from the mean responses of each of five or six preparations.

TABLE VII

Effects of gamma-hydroxybutyrate (GHB) on some membrane electrical properties of frog sartorius muscle fibres. The muscles were exposed to various concentrations of GHB for 30 minutes before the measurements were obtained.

Muscles (No.)	Fibres (No.)	Gamma-hydroxybutyrate Concentration (X 10^{-3} g/ml)	Resting Potential* (mV)	Threshold Depolarization (mV)	Threshold Current (X 10^{-7} Amp.)	Effective Resistance (K.Ω)	τ_m (msec)
3	25	0	88.9 ± 1.4	25.1 ± 1.4	1.6 ± 0.06	256 ± 8	11.5 ± 0.4
2	16	5	90.8 ± 1.1	27.9 ± 0.8	1.6 ± 0.07	259 ± 6	12.1 ± 0.5
2	13	10	91.5 ± 1.9	32.5 ± 1.1**	1.7 ± 0.08	266 ± 9	13.6 ± 0.4
3	14	15	86.2 ± 1.5	37.8 ± 1.3**	1.8 ± 0.05**	274 ± 6**	15.2 ± 0.5**

* Mean ± S.E., derived from mean values for each muscle.

** Treatment means are significantly different from the control means ($P < 0.05$).

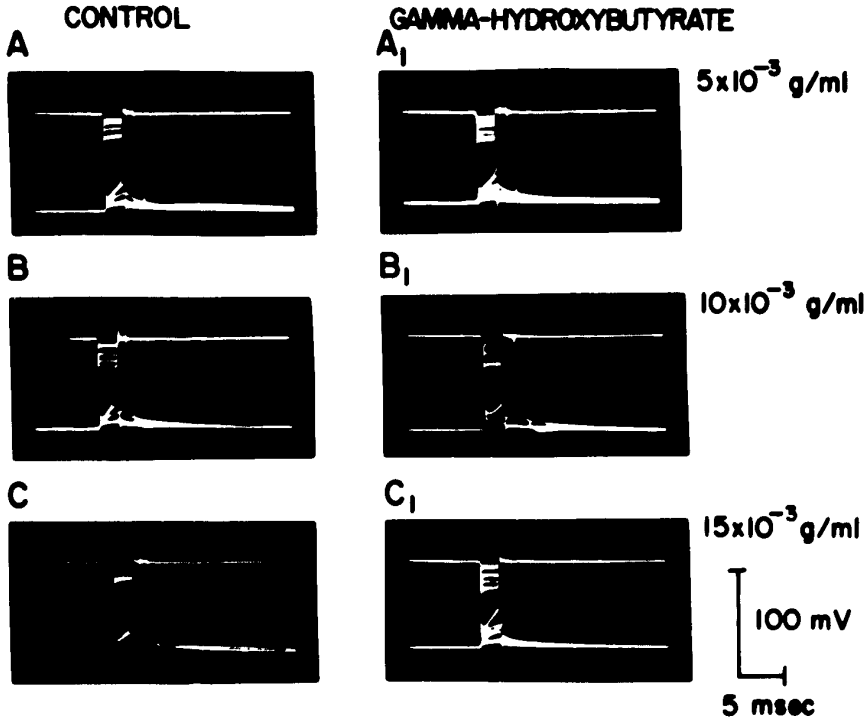

Figure 42. Effect of gamma-hydroxybutyrate on the intracellularly recorded action potentials from the frog sartorius muscle fibres. A, B, and C, are the control responses from separate fibres of the same muscle. A_1, B_1, and C_1, are the recordings done after 30 minutes exposure to a specified concentration of gamma-hydroxybutyrate. Upper traces show stimulus current records, lower traces indicate the passive electrotonic depolarizations and the action potentials produced by 2 msec current pulses.

of GHB were added to the Ringer's solution. Following the immersion of the fibres into 15 x 10^{-3} g/ml GHB, the action potential often failed to reach the zero potential base line (Figure 42-C_1), and in 4 fibres out of 14 examined an action potential could not be initiated by electrical stimulation. After the application of 15 x 10^{-3} g/ml GHB, there appeared a considerable prolongation of the action potential duration, while the peak of the spike became broad instead of being cusped and sharp as observed in the untreated fibres (compare records C and C_1 in Figure 42).

iii. Effect on the Current-Voltage Relation. Typical records regarding the effect of different concentrations of gamma-hydroxybutyrate on the current-voltage relation are shown in Figure 43. The same linear regression line was drawn through all the four graphs. Treating the muscles with 5 and 10 x 10^{-3} g/ml GHB did not alter the slope of this linear relation, from which it might be deduced that the passive membrane conductance remained almost unchanged. However, 15 x 10^{-3} g/ml GHB produced a counterclockwise rotation of the curve. Since GHB (15 x 10^{-3} g/ml) produced an appreciable increase of both the membrane time constant (τ_m), and the effective membrane resistance (Table VII) such a rotation of the curve is most likely due to the increase of the latter. Figure 43 also shows that the application of 10 and 15 x 10^{-3} g/ml GHB tends to suppress the local response, because most of the points following GHB treatment fall on the linear regression line as compared with the untreated controls.

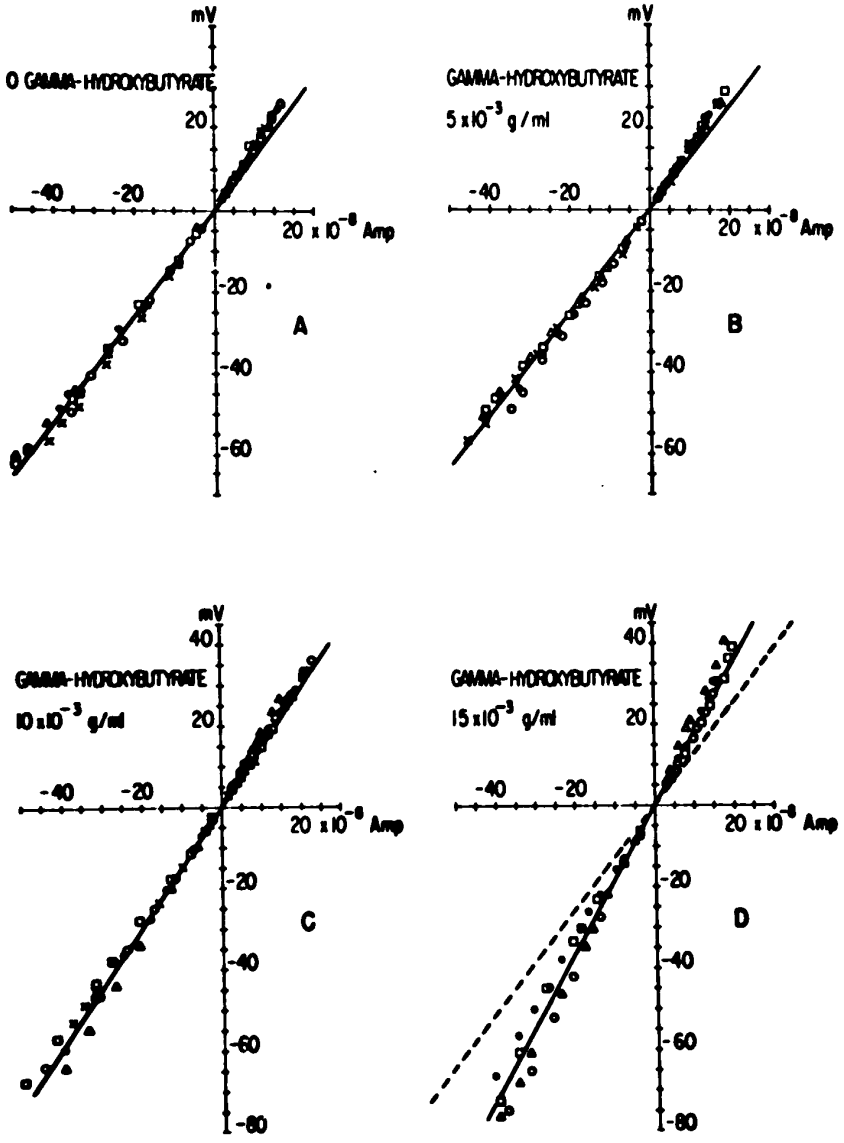

Figure 43. Effects of gamma-hydroxybutyrate treatment on the current-voltage relation of frog sartorius muscle fibres. The different symbols on each graph represent individual muscle fibres. A, indicates the control responses obtained from separate fibres of the same muscle in Ringer's solution. B, C, and D, are the responses recorded after 30 minutes exposure to gamma-hydroxybutyrate. The solid linear regression lines were drawn to fit the data obtained at the specified concentration. The broken line represents the control linear regression line (gamma-hydroxybutyrate = 0) superimposed to show the effects of gamma-hydroxybutyrate treatment. The potentials were measured at the end of 2 msec current pulses of the specified intensity.

iv. Effect on the Maximum Rate of Rise of the Action Potential. Figure 44 illustrates the typical differentiated action potentials recorded from single cells with an intracellular microelectrode while the fibres were stimulated by means of extracellular pore electrode. The data obtained from several fibres of three muscles kept in Ringer's solution having various concentrations of sodium and 15 x 10^{-3} g/ml GHB are shown in Figure 45. These results suggest a competitive inhibition, with the effect of 15 x 10^{-3} g/ml GHB being approximately equivalent to that of a 50% reduction in the external sodium concentration. The GHB-induced decrease in the maximum rate of rise of the spike can largely be overcome by increasing the amount of Na^{+} into the Ringer's fluid as revealed by record C_1 in Figure 44 and also by Figure 45. The other conspicuous change produced by GHB treatment is the reduction of the overshoot potential (Figure 46). Such an effect of GHB can also greatly be antagonized by the subsequent addition of sodium into the surrounding medium (Figure 46-C_1).

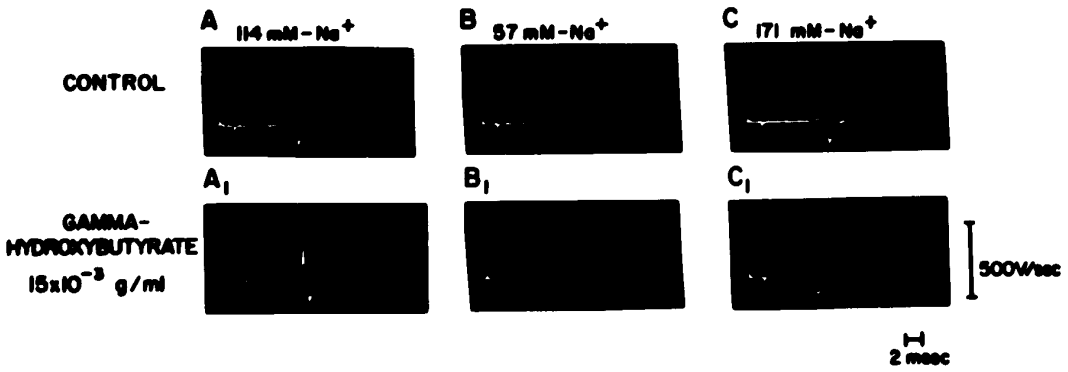

Figure 44. Differentiated, intracellularly recorded action potentials showing the effect of gamma-hydroxybutyrate on the maximum rate of rise. The fibres were stimulated with an extracellular pore electrode at a point distant from the recording electrode. The stimulus artifact appears at the start of each record.

The peak of each record represents the maximum rate of rise of the action potential under the specified condition. Responses were obtained from separate fibres of the same muscle. Sodium concentration of the bathing solution is shown above the records. The muscle was exposed to altered sodium and gamma-hydroxybutyrate concentrations for at least 30 minutes before each recording. A, B, and C, without gamma-hydroxybutyrate; A_1, B_1, and C_1, 15 x 10^{-3} g/ml gamma-hydroxybutyrate.

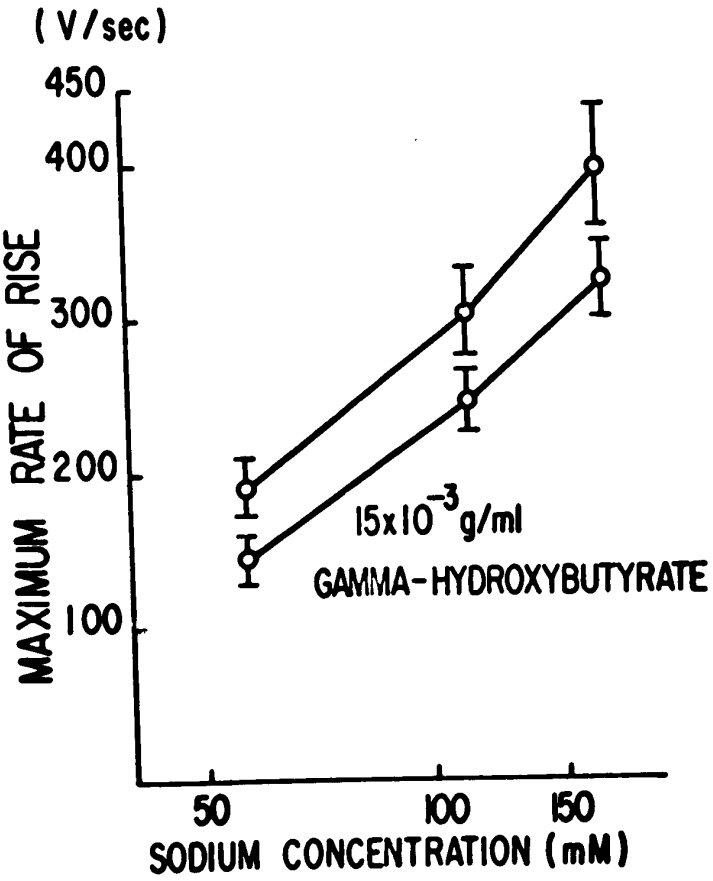

Figure 45. Effect of extracellular sodium concentration and of gamma-hydroxybutyrate on the maximum rate of rise of intracellularly recorded action potentials. Upper curve, Ringer's solution without gamma-hydroxybutyrate; lower curve, with gamma-hydroxybutyrate (15×10^{-3} g/ml). Mean and standard error derived from the mean values obtained in each of three separate preparations. Ordinate, maximum rate of rise of the action potential in V/sec; abscissa, sodium concentration of the bathing medium in mM.

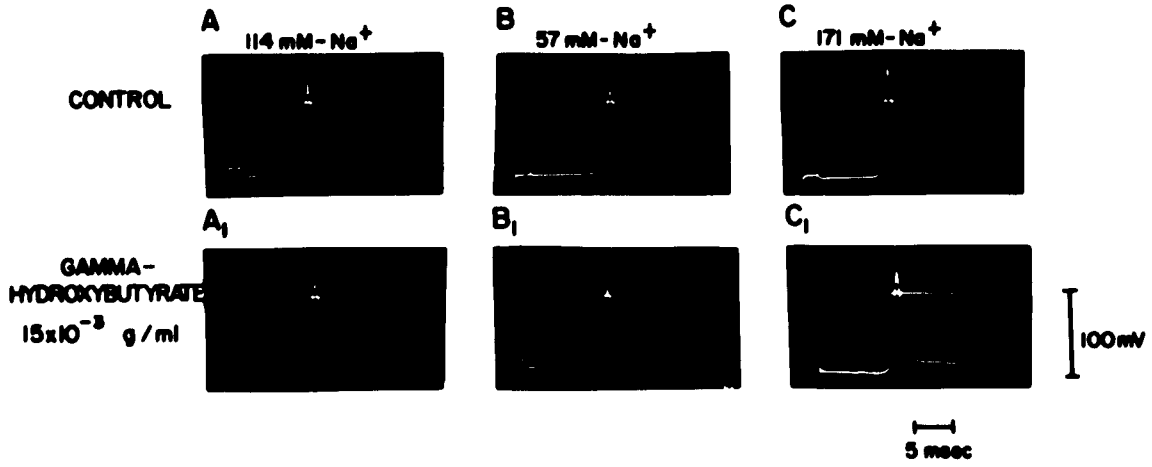

Figure 46. Effect of gamma-hydroxybutyrate on the overshoot of the intracellularly recorded action potentials. Sodium concentration of the bathing medium is indicated above the records. The stimulus artifact appears at the start of each action potential. The horizontal line across the upper part of all the records indicates zero potential.

Action potentials were recorded from separate fibres of the same muscle. The muscle was exposed to altered sodium and gamma-hydroxybutyrate concentrations for at least 30 minutes before each recording. A, B_2 and C, without gamma-hydroxybutyrate; A_1, B_1, and C_1, 15 x 10^{-3} g/ml gamma-hydroxybutyrate. In this muscle the mean overshoot potential (mV $\pm$ S.E.) under the various conditions was: A, 24.3 $\pm$ 2.6 (19 fibres); B, 14.5 $\pm$ 1.8 (10 fibres); C, 32.2 $\pm$ 2.3 (14 fibres); A_1, 19.6 $\pm$ 1.3 (8 fibres); B_1, 2.8 $\pm$ 2.1 (13 fibres); and C_1, 26.6 $\pm$ 1.5 (11 fibres).

(J) GAMMA-BUTYROLACTONE.

i. Studies with Extracellular Stimulation. The application of gamma-butyrolactone (GBL) to the sartorius muscle of the frog reversibly suppressed its excitability, and diminished the size of the compound action potential (Figure 47). Soaking the muscle bundle for 30 minutes in Ringer's solution containing 12.8×10^{-3} g/ml GBL caused a reduction of the action potential amplitude to about $52.1 \pm 3.3\%$ while the excitability was depressed to about $57.6 \pm 4.1\%$ of the control values. The recovery of responses from the highest concentration occurred after about 30 to 40 minutes following the removal of GBL from the muscle bath.

ii. Studies with Intracellular Electrodes. Figure 48 illustrates the superimposed records of the depolarizing subthreshold and threshold current pulses along with the simultaneously occurring voltage changes across the fibre membrane. Pre-treatment of the muscles with 6.4 and 12.8×10^{-3} g/ml of GBL markedly increased the amount of current needed for the initiation of the action potential. The threshold depolarization was increased, while the critical level of the membrane potential was slightly decreased under the influence of the increasing concentrations of gamma-butyrolactone. The other major change produced by GBL was the reduction, or the complete abolition of the overshoot potential. Quite often, the action potentials failed to reach the zero potential base line, and several of the fibres tested were rendered electrically inexcitable when 12.8×10^{-3}

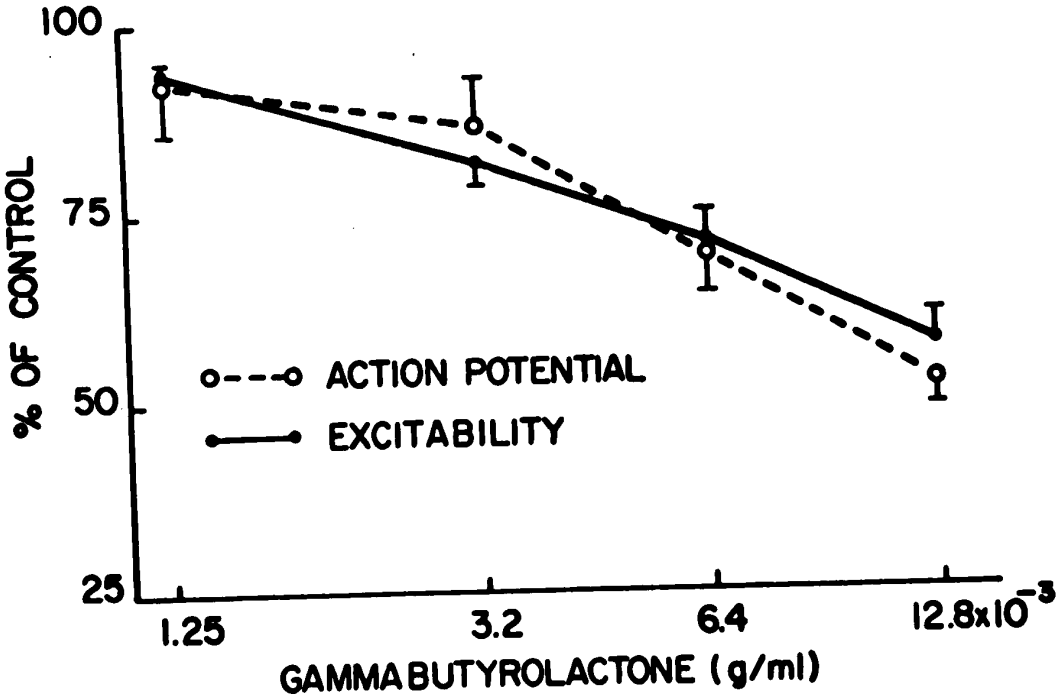

Figure 47. Effects of various concentrations of gamma-butyrolactone on the excitability, calculated as the inverse of the threshold current, and on the maximum size of the compound action potential of frog sartorius muscle strips. Each mean and standard error was calculated from the mean responses of each of five preparations.

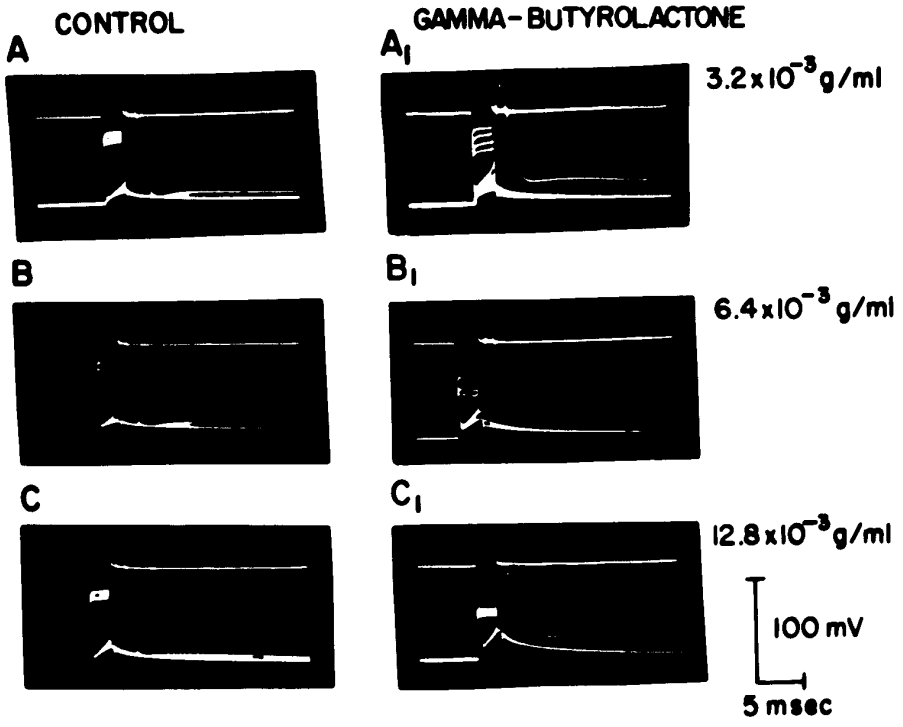

Figure 48. Effect of gamma-butyrolactone on the intracellularly recorded action potentials from the frog sartorius muscle fibres. A, B, and C, are the control responses from separate fibres of the same muscle. A_1, B_1, and C_1, are the recordings done after 30 minutes exposure to a specified concentration of gamma-butyrolactone. Upper traces show stimulus current records, while the lower traces represent the passive electrotonic depolarizations and the action potentials produced by 2 msec current pulses.

g/ml GBL were added to the Ringer's fluid. Records B_1 and C_1 of Figure 48 clearly demonstrate that the duration of the action potential was considerably prolonged, and the peak of the spike appeared broad following the immersion of the muscle into Ringer's solution containing 6.4 and 12.8 x 10^{-3} g/ml of gamma-butyrolactone. These observations indicate that both the rate of rise and the rate of fall of the action potential are considerably decreased by GBL treatment.

The results of the experiments performed with dual microelectrodes in the same muscle cell are listed in Table VIII. The excitability of the membrane was decreased with increasing concentrations of GBL, as was indicated by the increased threshold depolarization, and the concomitantly increased threshold current required to generate a propagated action potential. Nevertheless, no significant difference was observed in the resting potentials among the control fibres and those exposed to different concentrations of GBL.

iii. Effect on the Current-Voltage Relation. Gamma-butyrolactone treatment brought about no significant change in the slope of the linear regression line (Figure 49). These results pointed out that GBL did not appreciably influence the conductance in the passively functioning membrane. The absence of any marked variations in the τ_m, and in the effective membrane resistance among the GBL treated muscles and those immersed in ordinary Ringer's solution (Table VIII) strengthen the conclusion that the passive membrane conductance remains virtually unaltered by GBL treatment.

TABLE VIII

Effects of gamma-butyrolactone on some membrane electrical properties of frog sartorius muscle fibres. The muscles were soaked in various concentrations of gamma-butyrolactone for 30 minutes before the measurements were started.

Muscles (No.)	Fibres (No.)	Gamma-butyrolactone Concentration (X 10^{-3} g/ml)	Resting Potential* (mV)	Threshold Depolarization (mV)	Threshold Current (X 10^{-7} Amp.)	Effective Resistance (K.Ω)	τ_m (msec)
4	40	0	88.6 ± 1.4	23.4 ± 1.0	1.6 ± 0.08	258 ± 8	12.7 ± 0.8
4	26	3.2	89.1 ± 1.3	25.8 ± 1.2	1.8 ± 0.08	256 ± 7	12.2 ± 0.3
4	22	6.4	88.7 ± 1.1	28.1 ± 1.5**	2.3 ± 0.13**	259 ± 11	13.6 ± 0.4
3	16	12.8	90.1 ± 1.2	35.4 ± 1.4**	3.0 ± 0.14**	262 ± 9	14.2 ± 0.7

* Mean ± S.E., derived from the mean values for each muscle.

** Treatment means are significantly different from the control means ($P < 0.05$).

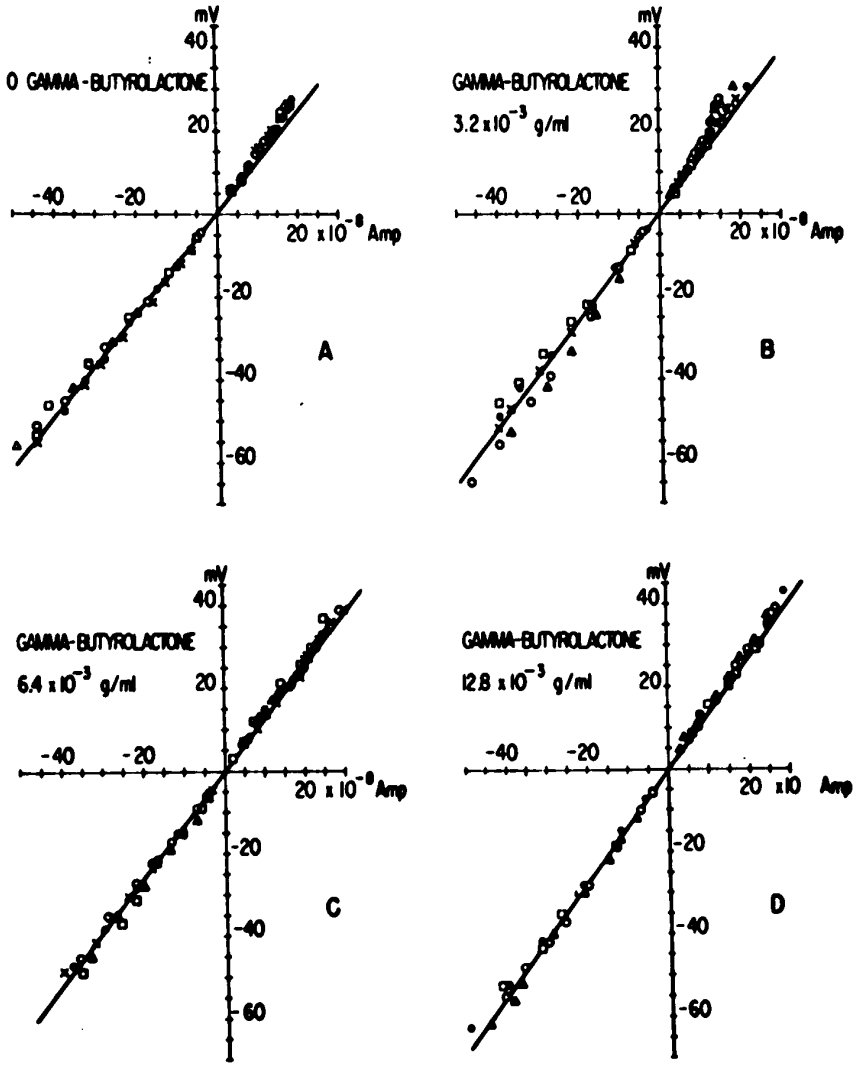

Figure 49. Effects of gamma-butyrolactone treatment on the current-voltage relation of frog sartorius muscle fibres. The different symbols on each graph represent individual muscle fibres. A, shows the control responses obtained from different fibres of the same muscle in Ringer's solution. B, C, and D, are the responses recorded after 30 minutes exposure to gamma-butyrolactone. The same linear regression line is drawn on all four graphs. The potentials were measured at the end of 2 msec current pulses of the specified intensity.

Nevertheless, the local potentials are suppressed following the application of 6.4 and 12.8 x 10^{-3} g/ml GBL (Figure 49-C and D), indicating a specific decrease of sodium conductance in the fibre membrane.

iv. Effect on the Maximum Rate of Rise of the Action Potential. Typical examples of the differentiated intracellularly recorded action potentials are shown in Figure 50. Treating the muscle with GBL had an effect similar to reducing the sodium concentration in the extracellular medium. The results obtained from four muscles are plotted in Figure 51. These results are indicative of a competitive inhibition, with the action of 12.8 x 10^{-3} g/ml GBL roughly equivalent to that of a 50% reduction in the external sodium concentration. Apart from decreasing the rate of rise, exposure to GBL reduced the overshoot of the action potential (Figure 52). Simultaneous addition of 171 mM sodium into the Ringer's solution antagonized the effect of GBL, and restored the size of the overshoot potential approximately equal to the level observed in normal Ringer's solution (compare records A and C_1 in Figure 52).

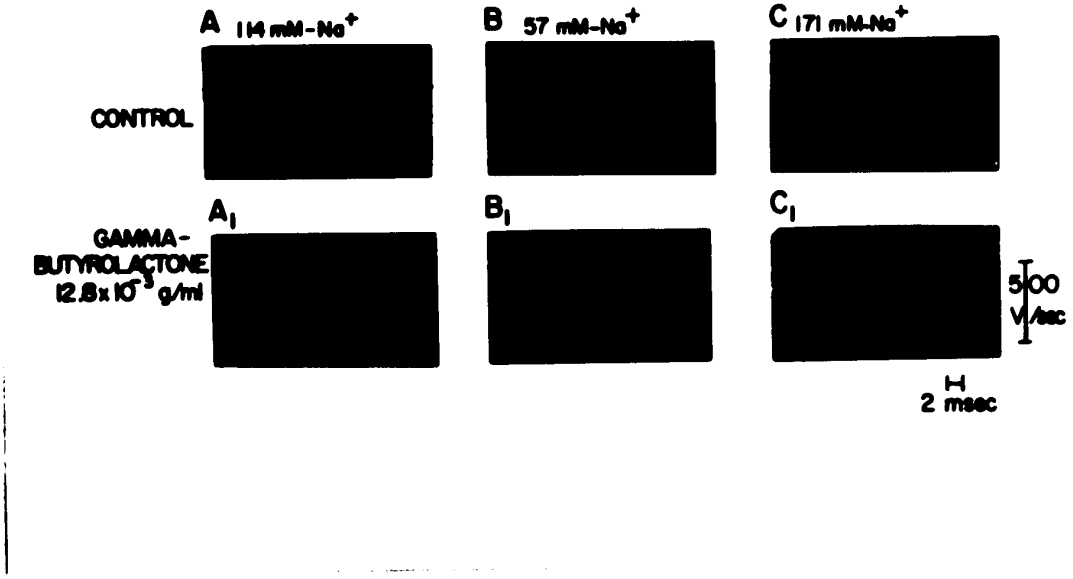

Figure 50. Differentiated, intracellularly recorded action potentials showing the effect of gamma-butyrolactone on the rate of rise of the action potential. The fibres were stimulated with an extracellular pore electrode at a point distant from the recording electrode. The stimulus artifact appears at the start of each record.

The peak of each record indicates the maximum rate of rise of the action potential under the specified concentration. Sodium concentration of the bathing medium is shown above the records. The muscle was exposed to altered sodium and gamma-butyrolactone concentration for 30 minutes before each recording. A, B, and C, without gamma-butyrolactone; A_1, B_1, and C_1, 12.8 x 10^{-3} g/ml gamma-butyrolactone.

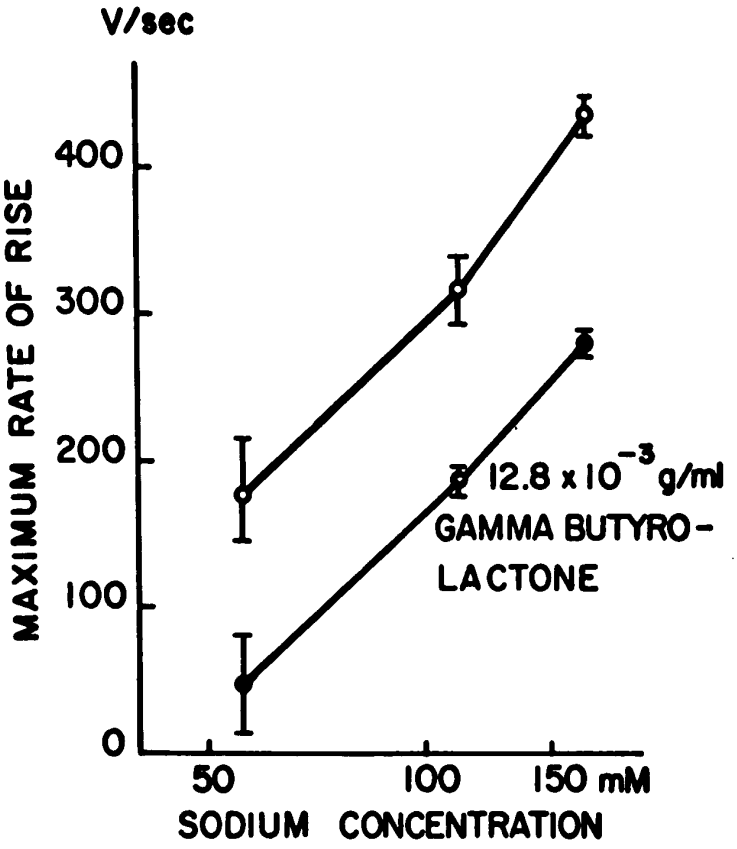

Figure 51. Effect of extracellular sodium concentration and of gamma-butyrolactone on the maximum rate of rise of intracellularly recorded action potentials. Upper curve, Ringer's solution without gamma-butyrolactone; lower curve, with gamma-butyrolactone (12.8×10^{-3} g/ml). Mean and standard error derived from the mean values obtained in each of four separate preparations. Ordinate, maximum rate of rise of the action potential in V/sec; abscissa, sodium concentration of the Ringer's solution in mM.

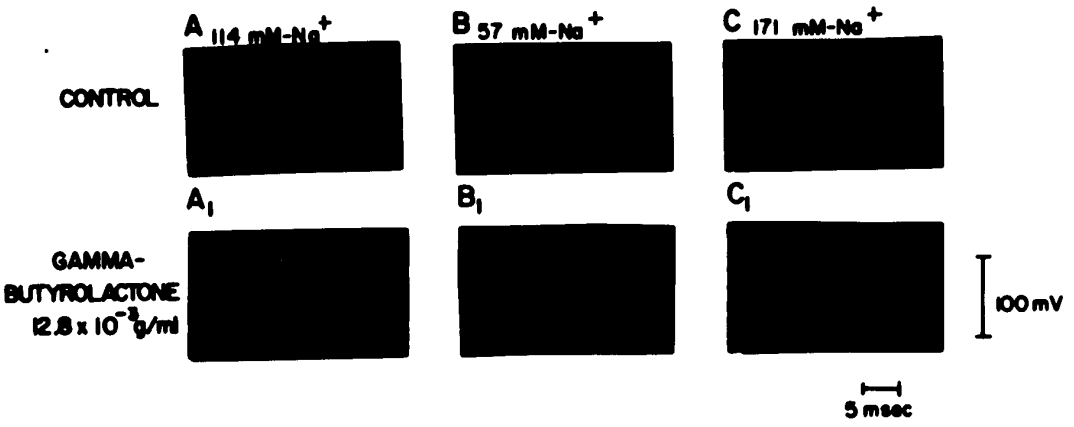

Figure 52. Effect of gamma-butyrolactone on the overshoot of the intracellularly recorded action potentials. Sodium concentration of the Ringer's solution is indicated above the records. The stimulus artifact appears at the beginning of each action potential. The horizontal line across the upper part of all the records indicates zero potential base line.

Action potentials were recorded from different fibres of the same muscle. The muscle was exposed to altered sodium and gamma-butyrolactone concentrations for 30 minutes before each recording. A, B, and C, without gamma-butyrolactone; A_1, B_1, and C_1, 12.8 x 10^{-3} g/ml gamma-butyrolactone. In this muscle the mean overshoot potential (mV $\pm$ S.E.) under the various conditions was: A, 27.2 $\pm$ 1.3 (23 fibres); B, 12.9 $\pm$ 1.7 (12 fibres); C, 35.7 $\pm$ 2.1 (16 fibres); A_1, 14.6 $\pm$ 1.5 (13 fibres); B_1, zero (10 fibres); and C_1, 24.7 $\pm$ 1.4 (12 fibres).

(K) GAMMA-AMINOBUTYRIC ACID:

i. Studies with Extracellular Stimulation. Figure 53 shows that the application of gamma-aminobutyric acid (GABA) in the range of 238.8 to 258.2 milliosmols/liter (0.5 to 2.5 x 10^{-3} g/ml) did not appreciably affect the maximum size of the compound action as well as the excitability of the muscle fibres. On the other hand, the action potential amplitude was reduced to about 55.2 $\pm$ 5.6%, and the excitability was depressed to about 62.8 $\pm$ 7.2% of the control level following the treatment with 428 milliosmols/liter (20 x 10^{-3} g/ml) of GABA. The recovery from the highest concentration of GABA was achieved after about 45 to 60 minutes interval following a 30 minutes exposure.

The addition of 20 x 10^{-3} g/ml GABA slightly decreased the pH of the normal Ringer's solution from about 7.6 to between 7.3 to 7.4. No correction of the pH was made for the reasons given previously. It may be noted from Figure 53 that the concentrations of GABA have been expressed in milliosmols rather than in g/ml. Such a scale was chosen to easily compare the effects of various concentrations of GABA with the iso-osmolar concentrations of sucrose on the excitability, and on the amplitude of the compound action potential under identical conditions. It may also be remarked, that the isotonic concentration of the normal Ringer's fluid was in the neighbourhood of 234 milliosmols/liter. The osmolarity of the solutions was frequently checked before and after the dissolution of the appropriate amounts of GABA into the Ringer's fluid.

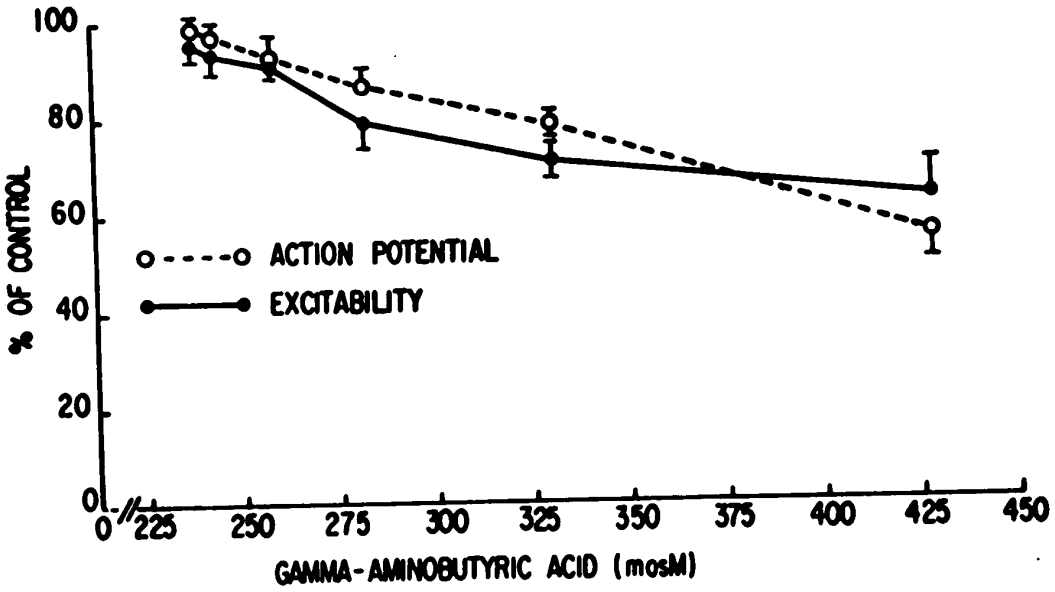

Figure 53. Effects of various concentrations of gamma-aminobutyric acid on excitability, calculated as the inverse of the threshold, and on the maximum size of the compound action potential of frog sartorius muscle strips. Each mean and standard error was calculated from the mean responses of each of six preparations.

ii. Studies with Intracellular Electrodes. Experiments with internal electrodes showed that following the exposure to GABA, the excitability of the fibre membrane was depressed, as indicated by both the increase in the threshold depolarization and the threshold current (Table IX). There was a tendency of the fibres treated with 10 and 20 x 10^{-3} g/ml GABA to be hyperpolarized by about 4 to 6 millivolts. The slight hyperpolarization of the fibre membrane in hypertonic solutions has been ascribed to the increased internal potassium concentration (Sperelakis and Schneider, 1968). It is likely that the resting potential changes produced by hyperosmotic solutions of GABA might have resulted from a higher internal potassium concentration, $[K^+]_i$.

Figure 54 illustrates the superimposed records of the passive electrotonic depolarizations and the action potentials produced by 2 msec current pulses of different intensities. Record C_1 clearly shows that GABA at a concentration of 20 x 10^{-3} g/ml (428 mosm.) markedly increased the threshold depolarization and decreased the firing level of the membrane as opposed to the fibres equilibrated in normal Ringer's solution. Increasing concentrations of GABA either reduced, or completely abolished the overshoot potential as shown in Figure 54. Associated with all these actions, there invariably occurred a prolongation of the action potential duration which became quite prominent after the treatment with 20 x 10^{-3} g/ml GABA (Figure 54). In many fibres the peak of the action potential was actually below the zero potential base line when the muscle was immersed in a solution con-

TABLE IX

Effects of gamma-aminobutyric acid (GABA) on some membrane electrical properties of frog sartorius muscle fibres. The muscles were exposed to various concentrations of GABA for 30 minutes before the measurements were obtained.

Muscles (No.)	Fibres (No.)	Gamma-aminobutyric acid Concentration (X 10^{-3} g/ml)	Resting Potential* (mV)	Threshold Depolarization (mV)	Threshold Current (X 10^{-7} Amp.)	Effective Resistance (K.Ω)	τ_m (msec)
5	28	0	89.6 ± 1.8	24.2 ± 0.7	1.5 ± 0.06	256 ± 10	11.7 ± 0.8
4	16	5 (282.5)†	90.4 ± 1.1	26.4 ± 0.9	1.6 ± 0.07	260 ± 8	12.4 ± 0.5
3	12	10 (331.0)	93.6 ± 1.5	29.5 ± 0.8**	1.9 ± 0.05**	280 ± 6**	13.6 ± 0.7**
3	18	20 (428.0)	95.8 ± 1.9	35.6 ± 1.9**	2.2 ± 0.15**	305 ± 9**	15.3 ± 1.0**

* Mean ± S.E., derived from mean values for each muscle.

† Figures in parentheses indicate the concentration in milliosmols/liter. (Ringer's solution plus GABA).

** Treatment means are significantly different from the control means ($P < 0.05$).

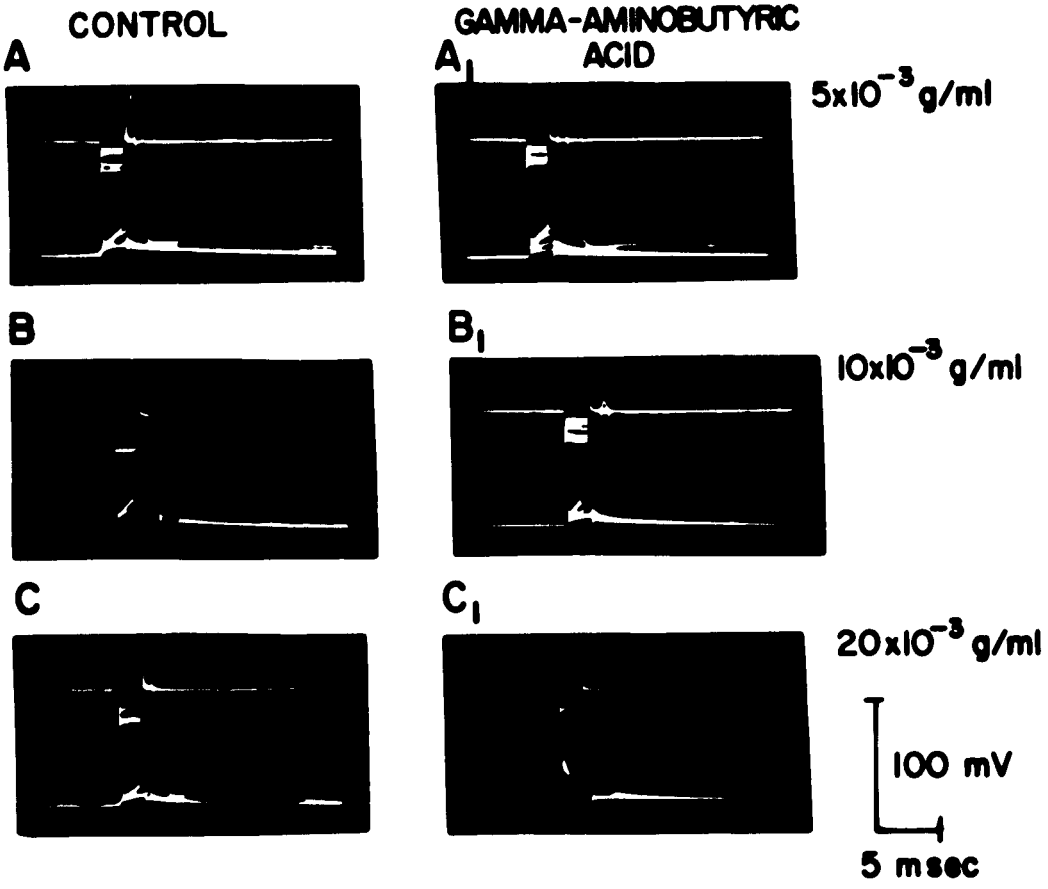

Figure 54. Effect of gamma-aminobutyric acid on the intracellularly recorded action potentials from the frog sartorius muscle fibres. A, B, and C, are the control responses from separate fibres of the same muscle. A_1, B_1, and C_1, are the recordings done after 30 minutes exposure to a specified concentration of gamma-aminobutyric acid. Upper traces show stimulus current records, lower traces indicate the passive electrotonic depolarizations and the action potentials produced by 2 msec current pulses.

taining 20 x 10^{-3} g/ml GABA; however, none of the fibres was rendered totally electrically inexcitable. In other words, even the highest concentrations of GABA did not prevent the initiation of the action potential. In contrast, gamma-hydroxybutyrate (as already described) at a relatively smaller concentration (15 x 10^{-3} g/ml) completely blocked the action potential production in about 29% of the fibres impaled. It therefore seems that gamma-hydroxybutyrate has a comparatively stronger depressant action on the frog muscle fibres than gamma-aminobutyric acid.

iii. Effect on the Current-Voltage Relation. Treatment of the muscle with 5 x 10^{-3} g/ml GABA did not alter the slope of the current-voltage curve, however, 10 x 10^{-3} g/ml GABA produced a small but definite counterclockwise rotation of the regression line (Figure 55). The magnitude of this rotation became more pronounced with 20 x 10^{-3} g/ml GABA as shown in Figure 55-D. Inspection of Table IX would indicate that increasing concentrations of GABA produced an increase in both the membrane time constant (τ_m), and in the effective membrane resistance. These observations are indicative of the fact that the counterclockwise shift of the curves is most probably due to an increase in the effective membrane resistance between the inside and the outside of the fibre membrane at the point of stimulation. These observations suggest that the addition of GABA in a concentration range of 10 x 10^{-3} g/ml into the Ringer's fluid causes a suppression of the passive membrane conductance.

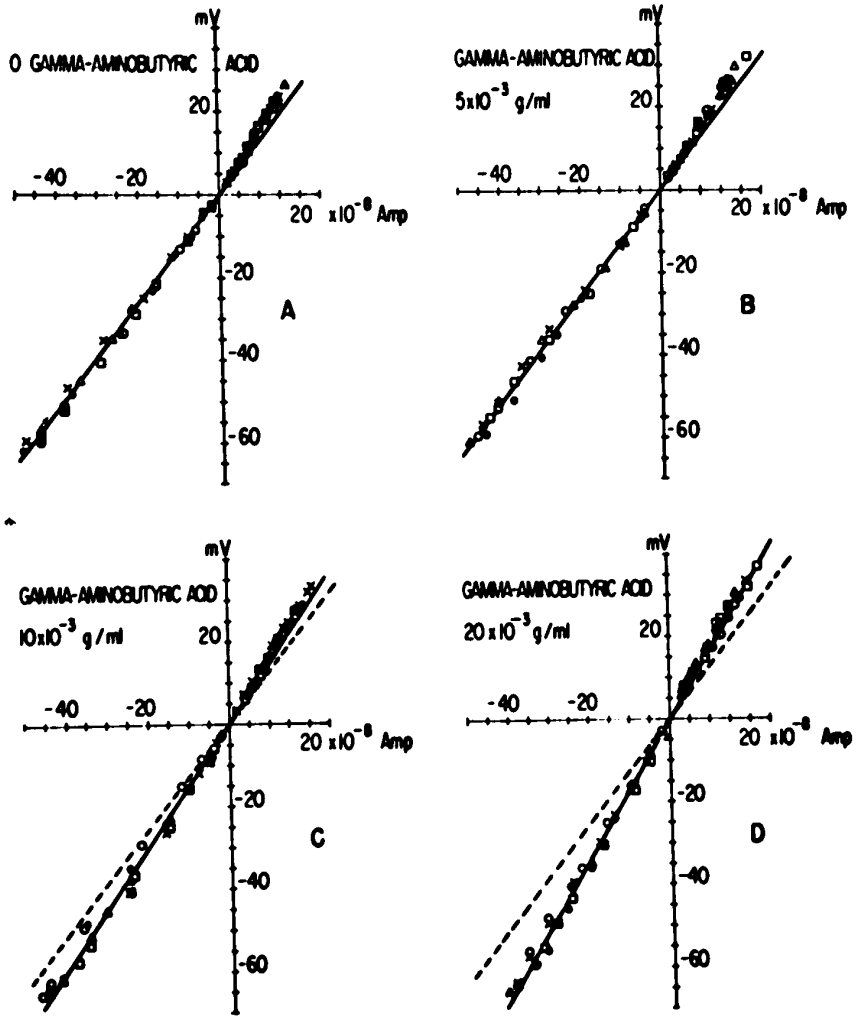

Figure 55. Effects of gamma-aminobutyric acid treatment on the current-voltage relation of frog sartorius muscle fibres. The different symbols on each graph represent individual muscle fibres. A, indicates the control responses obtained from separate fibres of the same muscle in Ringer's solution. B, C, and D, are the responses recorded after 30 minutes exposure to gamma-aminobutyric acid. The solid linear regression lines were drawn to fit the data obtained at the specified concentration. The broken lines represent the control linear regression line (gamma-aminobutyric acid = 0) superimposed to show the effects of gamma-aminobutyric acid treatment. The potentials were measured at the end of 2 msec current pulses of the specified intensity.

Figure 55-C and D also show that pre-treatment of the fibres with GABA greatly diminishes or completely eliminates the local potential production, since almost all points fall on the linear regression line following the treatment with 10 and 20 x 10^{-3} g/ml GABA. These results indicate that the application of GABA in high enough concentrations suppresses the increase in the sodium conductivity of the skeletal muscle membrane which produces the local potential (Hodgkin, 1938).

iv. Effect on the Maximum Rate of Rise of the Action Potential. Typical examples of the differentiated intracellularly recorded action potentials are shown in Figure 56. Treating the muscles with GABA had an effect similar to that of reducing the external sodium concentration. The data obtained from four muscles are plotted in Figure 57 against the sodium concentration in the bathing medium. When the muscle was soaked into Ringer's solution containing 20 x 10^{-3} g/ml GABA and 171 mM sodium (tonicity of such a solution was approximately 526 milliosmols/liter) the amplitude of the differentiated action potential was markedly reduced (Figure 56-C_1), instead of increased as observed with all drugs previously described. The maximum rate of rise of the action potential became approximately equivalent to that seen with a 50% reduction in the external sodium concentration (Figure 57), following the addition of GABA (20 x 10^{-3} g/ml) into Ringer's solution (171 mM - Na^+). Similarly, dissolution of 20 x 10^{-3} g/ml GABA into Ringer's fluid containing elevated sodium

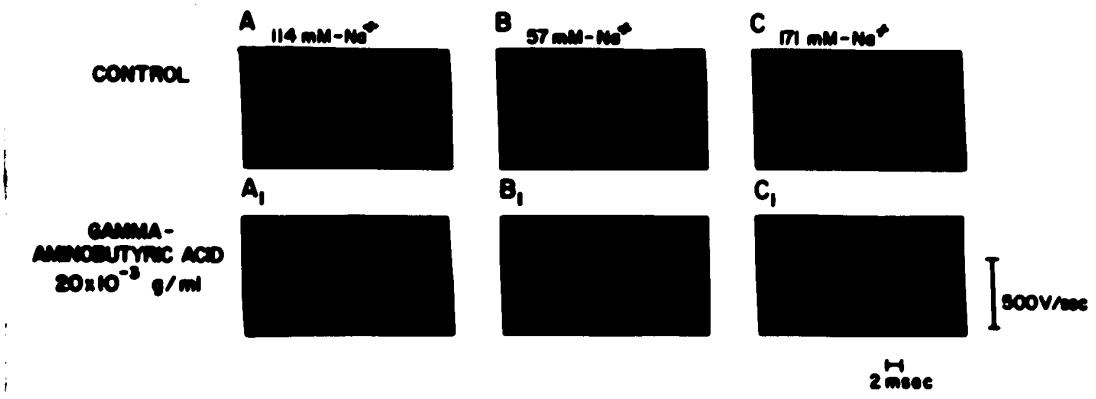

Figure 56. Differentiated intracellularly recorded action potentials showing the effect of gamma-aminobutyric acid on the maximum rate of rise. The fibres were stimulated with an extracellular pore electrode at a point distant from the recording electrode. The stimulus artifact appears at the start of each record.

The peak of each record represents the maximum rate of rise of the action potential under the specified condition. Responses were obtained from separate fibres of the same muscle. Sodium concentration of the bathing solution is shown above the records. The muscle was exposed to altered sodium and gamma-aminobutyric acid concentrations for at least 30 minutes before each recording. A, B, and C, without gamma-aminobutyric acid; A_1, B_1, and C_1, 20 x 10^{-3} g/ml gamma-aminobutyric acid.

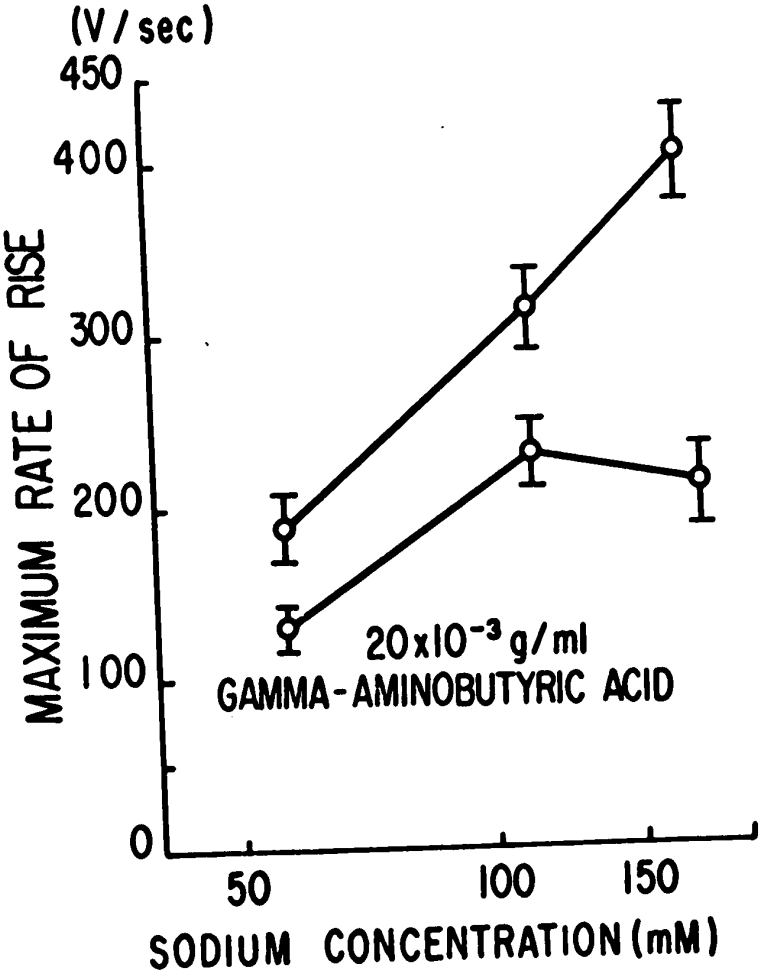

Figure 57. Effect of extracellular sodium concentration and of gamma-aminobutyric acid on the maximum rate of rise of intracellularly recorded action potentials. Upper curve, Ringer's solution without gamma-aminobutyric acid; lower curve, with gamma-aminobutyric acid (20 x 10^{-3} g/ml). Mean and standard error derived from the mean values obtained in each of four separate preparations. Ordinate, maximum rate of rise of the action potential in V/sec; abscissa, sodium concentration of the bathing medium in mM.

(171 mM) caused a marked depression of the overshoot potential (Figure 58-C_1). In this respect the results produced by GABA are entirely opposite to rest of the drugs reported in the present study, because increasing the extracellular sodium concentration did not antagonize the effect of GABA, and instead produced an additional depression in both the maximum rate of rise and the overshoot of the action potential. Evidence will be presented later to indicate that the GABA-induced depression of the excitability in the frog's skeletal muscle is most probably due to the hyperosmolarity (hypertonicity) of the solutions used rather than the narcotic action of GABA on the fibre membrane; since iso-osmolar concentrations of a pharmacologically inert substance like sucrose can also similarly suppress the responses under investigation.

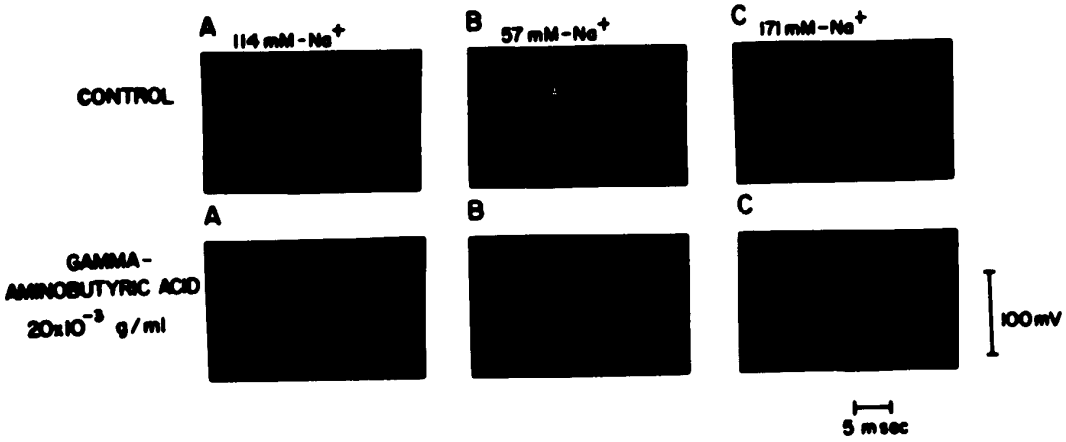

Figure 58. Effect of gamma-aminobutyric acid on the overshoot of the intracellularly recorded action potentials. Sodium concentration of the bathing medium is indicated above the records. The stimulus artifact appears at the start of each action potential. The horizontal line across the upper part of all the records indicates zero potential.

Action potentials were recorded from separate fibres of the same muscle. The muscle was exposed to altered sodium and gamma-aminobutyric acid concentrations for at least 30 minutes before each recording. A, B, and C, without gamma-aminobutyric acid; A_1, B_1, and C_1, 20×10^{-3} g/ml gamma-aminobutyric acid. In this muscle the mean overshoot potential (mV $\pm$ S.E.) under the various conditions was: A, 26.8 $\pm$ 1.8 (26 fibres); B, 11.8 $\pm$ 1.6 (10 fibres); C, 36.3 $\pm$ 2.5 (12 fibres); A_1, 18.7 $\pm$ 1.4 (8 fibres); B_1, 2.3 $\pm$ 1.9 (11 fibres); and C_1, 20.6 $\pm$ 2.8 (13 fibres).

(L) SUCROSE:

i. Studies with Extracellular Stimulation. The object of these experiments was to compare the effects of GABA with iso-osmolar concentrations of sucrose on the isolated sartorius muscle of the frog. The results of the osmotically-induced depression of the excitability and of the maximum size of the compound action potential are summarized in Figure 59. Soaking the muscle for 30 minutes in Ringer's solution containing sucrose ranging from about 238.4 to 258.2 millios-mols/liter (1.66 to 8.3 x 10^{-3} g/ml) scarcely affected both the excitability and the height of the compound action potential. However, these responses were progressively decreased with higher concentrations of sucrose; and a concentration of about 428 mosm. (66.4 x 10^{-3} g/ml) caused a decrease in the size of the compound action potential to about 60.2 $\pm$ 6.8%, and suppressed the excitability to about 70.5 $\pm$ 7.1% of the control values. The recovery from the highest concentration of sucrose occurred after about 30 to 45 minutes washing of the muscle bundle in Ringer's fluid. These results are comparable to those obtained with iso-osmolar concentrations of GABA and provide a basis for comparison with results obtained by using intracellular micro-electrodes under similar experimental conditions.

ii. Studies with Intracellular Electrodes. Table X is a summary of the experiments on muscles kept in hypertonic sucrose solutions. The significant change is the depression of the excitability, as indicated by the increase of the threshold depolarization

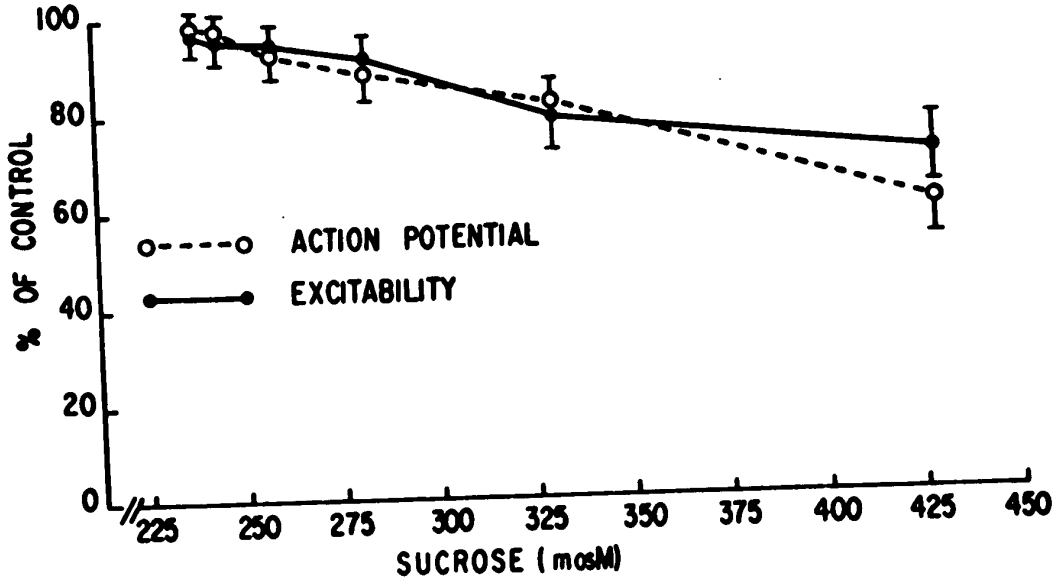

Figure 59. Effects of various concentrations of sucrose on excitability, calculated as the inverse of threshold, and on the maximum amplitude of the compound action potential of frog sartorius muscle strips. Each mean and standard error was calculated from the mean responses of each of five preparations.

TABLE X

Effects of hypertonic solutions of sucrose on some membrane electrical properties of frog sartorius muscle fibres. The muscles were exposed to various concentrations of sucrose for 30 minutes before the measurements were obtained.

Muscles (No.)	Fibres (No.)	Sucrose Concentration (X 10^{-3} g/ml)	Resting Potential* (mV)	Threshold Depolarization (mV)	Threshold Current (X 10^{-7} Amp.)	Effective Resistance (K.Ω)	τ_m (msec)
3	24	0	90.3 ± 1.1	23.4 ± 0.8	1.4 ± 0.05	254 ± 8	11.2 ± 0.6
3	13	16.6 (282.4)†	91.8 ± 1.2	26.6 ± 0.9	1.5 ± 0.08	263 ± 6	12.7 ± 0.4
3	14	33.2 (331.0)	92.3 ± 1.1	28.7 ± 0.9**	1.7 ± 0.06**	284 ± 9**	14.8 ± 0.8**
2	9	66.4 (428.0)	94.6 ± 1.8	33.0 ± 1.2**	1.8 ± 0.05**	298 ± 7**	15.4 ± 0.6**

* Mean ± S.E., derived from mean values for each muscle.

† Figures in parentheses indicate the concentration in milliosmols/liter. (Ringer's solution plus sucrose)

** Treatment means are significantly different from the control means ($P < 0.05$).

and the threshold current. Although there was no significant difference in the resting membrane potential between the control muscles and those immersed into Ringer's fluid containing various amounts of sucrose yet there was a tendency for the fibres in the hypertonic solutions to be hyperpolarized by a few millivolts (Table X). The slight increase in the resting potential in hypertonic solution made by the addition of sucrose in frog Ringer's solution has been attributed to the higher internal potassium concentration (Freygang et al., 1964; Sperelakis and Schneider, 1968).

Figure 60 illustrates the superimposed records of the depolarizing current pulses and the accompanying changes in the membrane potential before and after immersing the muscle in hypertonic sucrose solutions. Inspection of record C_1 would clearly indicate that the threshold depolarization was increased and the critical level of the membrane potential was slightly decreased by the addition of 66.4×10^{-3} g/ml sucrose (about 428 mosm.; approximately 1.83 times the osmolarity of normal Ringer's solution). The threshold increase was not maintained after the muscle was returned to Ringer's solution, i.e. the effect of sucrose was completely reversible. Thus the changes produced by hypertonic solutions of sucrose fitted those data obtained after the treatment with iso-osmolar concentrations of GABA. This agreement between the changes produced by GABA and sucrose suggests that the depression of the excitability produced by GABA does not seem to be due to its narcotic action on the skeletal muscle, but appears to be due to the hyperosmotic effect on the fibre membrane.

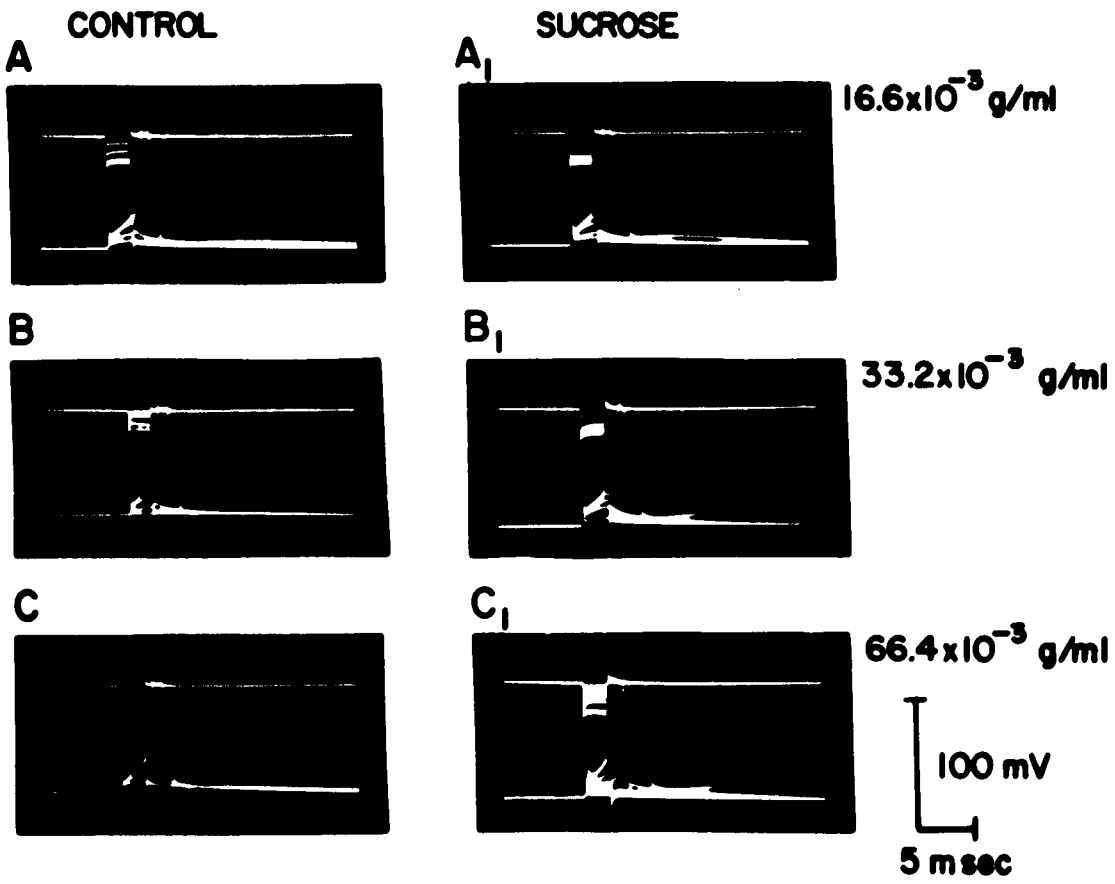

Figure 60. Effect of hypertonic solutions of sucrose on the intracellularly recorded action potentials from the frog sartorius muscle fibres. A, B, and C, are the control responses from separate fibres of the same muscle . A_1, B_1, and C_1, are the recordings done after 30 minutes exposure to a specified concentration of sucrose. Upper traces, show stimulus current records, lower traces indicate the passive electrotonic depolarizations and the action potentials produced by 2 msec current pulses.

iii. Effect on the Current-Voltage Relation. As shown in Figure 61 the current-voltage relation remained essentially unaffected after the addition of 16.6×10^{-3} g/ml of sucrose (about 282.4 mosm.) into the Ringer's fluid. In contrast, treatment with 33.2×10^{-3} g/ml sucrose (about 331 mosm.) produced a small counterclockwise rotation of the regression line, and the amount of this rotation was significantly increased with 66.4×10^{-3} g/ml sucrose (about 428 mosm.). Reference to Table X would indicate that increasing concentrations of sucrose produced a progressive increase in both the effective membrane resistance and the membrane time constant (τ_m). These additional observations suggest that the counterclockwise rotation of the current-voltage curves is most likely as a consequence of the increased effective membrane resistance. These observations point out that like GABA, the iso-osmolar concentrations of sucrose can also cause a decrease in the passive membrane conductance. Furthermore, pretreatment of the fibres with hypertonic solutions of sucrose either greatly diminished or completely abolished the local response (Figure 61-C and D). These results show that the increase in the membrane sodium conductivity specifically responsible for the production of the local response is also suppressed under the influence of the hyperosmotic solutions.

iv. Effect on the Maximum Rate of Rise of the Action Potential. Typical recordings of the differentiated action potentials prior to and after the treatment with 66.4×10^{-3} g/ml sucrose are shown in Figure 62. Treating the muscle with hypertonic solutions had

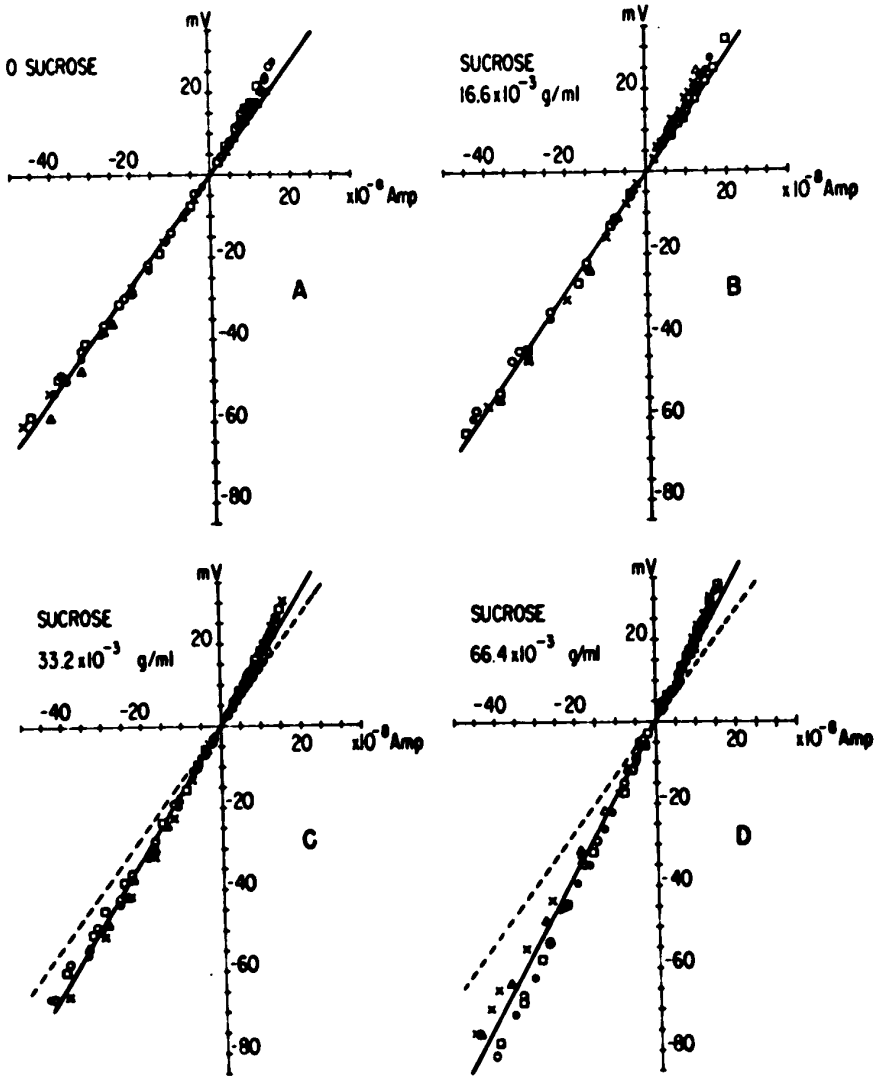

Figure 61. Effects of sucrose treatment on the current-voltage relation of frog sartorius muscle fibres. The different symbols on each graph represent individual muscle fibres. A, indicates the control responses obtained from separate fibres of the same muscle in Ringer's solution. B, C, and D, are the responses recorded after 30 minutes exposure to sucrose. The solid linear regression lines were drawn to fit the data obtained at the specified concentration. The broken lines represent the control linear regression lines (sucrose = 0) superimposed to show the effects of sucrose treatment. The potentials were measured at the end of 2 msec current pulses of the specified intensity.

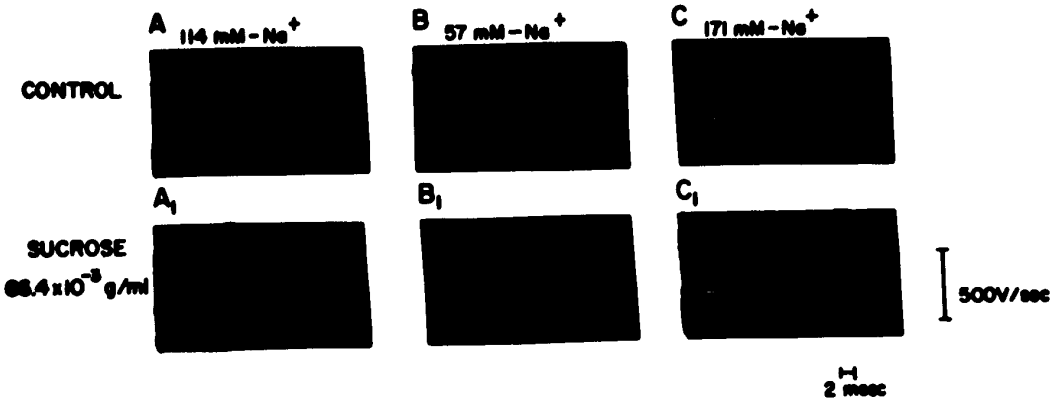

Figure 62. Differentiated, intracellularly recorded action potentials showing the effect of hypertonic solutions of sucrose on the maximum rate of rise. The fibres were stimulated with an extracellular pore electrode at a point distant from the recording electrode. The stimulus artifact appears at the start of each record.

The peak of each record represents the maximum rate of rise of the action potential under the specified condition. Responses were obtained from separate fibres of the same muscle. Sodium concentration of the bathing solution is shown above the records. The muscle was exposed to altered sodium and sucrose concentrations for at least 30 minutes before each recording. A, B, and C, without sucrose; A_1, B_1, and C_1, 66.4 x 10^{-3} g/ml sucrose.

an effect similar to reducing the external sodium concentration. The results obtained in four experiments of this type are plotted on the graph shown in Figure 63. Exposing the muscle fibres to a solution containing 171 mM sodium and 66.4 x 10^{-3} g/ml sucrose (tonicity of this solution was about 530 milliosmols/liter) decreased the maximum rate of rise of the action potential; the magnitude of the latter became approximately equivalent to that observed with a 50% reduction in the extracellular sodium concentration (Figure 63). The overshoot potential was also decreased by the presence of 66.4 x 10^{-3} g/ml sucrose and 1.5 times the usual concentration of sodium (171 mM instead of 114 mM) in the bathing fluid as shown by Figure 64. Since all the effects of GABA on the maximum rate of rise and the overshoot of the action potential can be duplicated by iso-osmolar concentrations of sucrose, hence it is suggested that most, if not all, of the GABA-induced depression of the excitability in the frog's sartorius muscle is owing to the hyperosmotic action of the solutions on the fibre membrane and not due to the anesthetic effect of the drug as <u>per se</u>.

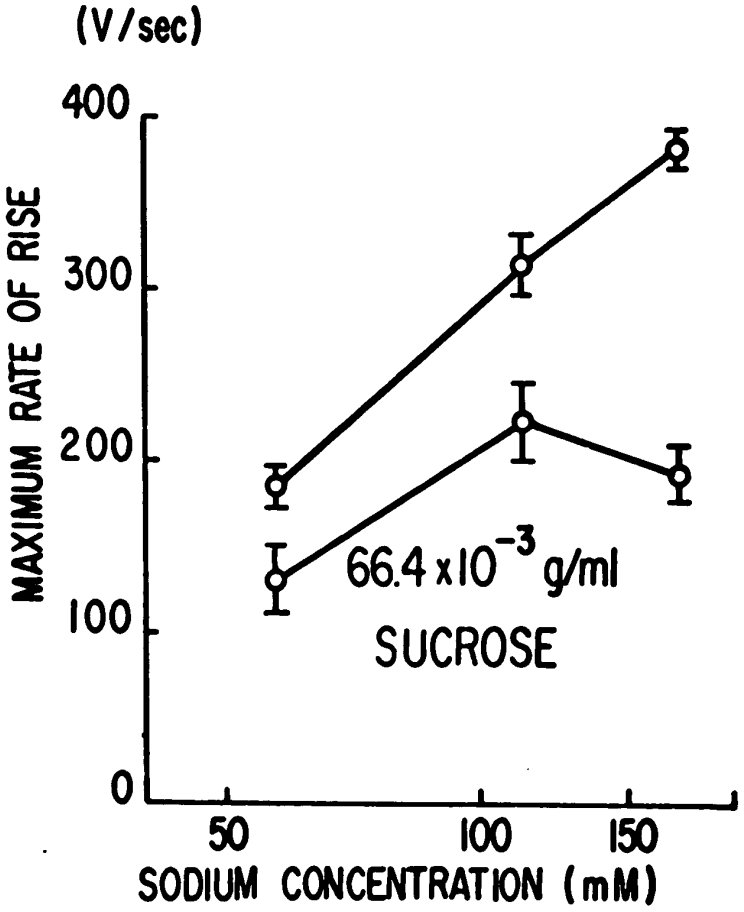

Figure 63. Effect of extracellular sodium concentration and of sucrose on the maximum rate of rise of the intracellularly recorded action potentials. Upper curve, Ringer's solution without sucrose; lower curve, with sucrose (66.4 x 10^{-3} g/ml). Mean and standard error derived from the mean values obtained in each of three separate preparations. Ordinate, maximum rate of rise of the action potential in V/sec; abscissa, sodium concentration of the bathing medium in mM.

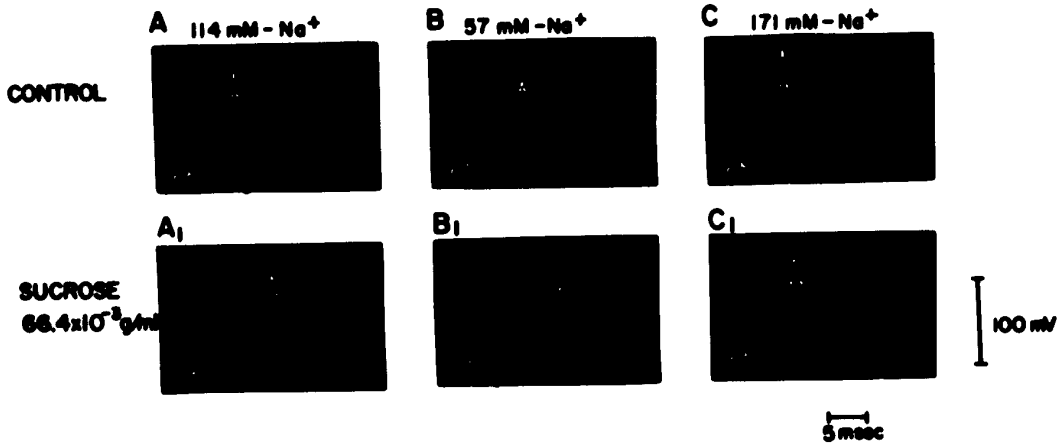

Figure 64. Effect of hypertonic solutions of sucrose on the overshoot of the intracellularly recorded action potentials. Sodium concentration of the bathing medium is indicated above the records. The stimulus artifact appears at the beginning of each action potential. The horizontal line across the upper part of all the records indicates zero potential.

Action potentials were recorded from separate fibres of the same muscle. The muscle was exposed to altered sodium and sucrose concentrations for at least 30 minutes before each recording. A, B, and C, without sucrose; A_1, B_1, and C_1, 66.4 x 10^{-3} g/ml sucrose. In this muscle the mean overshoot potential (mV $\pm$ S.E.) under the various conditions was: A, 29.7 + 2.1 (29 fibres); B, 14.2 + 1.8 (12 fibres); C, 38.6 + 2.2 (14 fibres); A_1, 22.0 + 1.7 (9 fibres); B_1, 1.6 $\pm$ 2.7 (8 fibres); and C_1, 24.4 $\pm$ 1.9 (12 fibres).

CHAPTER V

DISCUSSION

CHAPTER V. DISCUSSION.

Although a number of *in vivo* techniques are used for the screening and evaluation of compounds for local and general anesthetic activity, direct electrophysiological investigations of the electrical properties of the isolated muscle, nerve or cerebral cortex have considerable advantages. For example, the modification of the electrophysiological measurements following the application of depressant drugs to isolated preparations not only allow more precise comparisons of the potencies of a variety of different compounds, but can also help to elucidate the mechanism involved in the conduction block. Another advantage of the isolated preparations is that they permit investigations of the physical chemical factors such as the effect of alteration in pH on the ionization of the anesthetic solution, and the active moiety of the anesthetic molecule (cation or anion) responsible for the blockade of impulse transmission (Skou, 1954; Ritchie and Greengard, 1961). The classical methods used for the evaluation of the effectiveness of the local and general anesthetics require a rather large number of determinations for reasonable accuracy and the subjective nature of some of these tests is apt to be source of error (Bennett *et al.*, 1942). Nevertheless, tests of local tissue reactions and general toxicity in the intact animals are invariably required before any local or general anesthetic, or a general central nervous system depressant drug can be declared safe for therapeutic use. The

importance of the isolated nerve and nerve muscle preparations for testing and evaluation of local anesthetics has been stressed by Camougis and Takman (1971).

In the Results section, the effects produced by each drug on the passive and active properties of the muscle membrane have been dealt under different sub-headings and are quite descriptive. Therefore the changes noticed in the excitability will be briefly discussed and the supportive evidence from the literature for such changes will be sought. It may, however, be remarked in the beginning that the subsequent interpretation will apply to all the drugs used in this study except GABA, because the mechanism of GABA-induced depression of excitability seems to be different than of the other drugs.

Studies with extracellular electrodes showed that increasing concentrations of chlorpromazine, promethazine, diphenhydramine, scopolamine, meperidine, morphine, gamma-hydroxybutyrate and gamma-butyrolactone caused a progressive depression of the amplitude of the compound action potential and the excitability of the muscle fibres. The intracellular recordings from individual cells also showed that all these drugs produced a depression in the membrane excitability, as indicated by both the increase of threshold depolarization and the threshold current required to generate a propagated action potential. The resting membrane potential however remained almost unchanged. There is now ample amount of evidence to suggest that the onset of the electrical activity in nerve and skeletal muscle depends upon a selective and momentary increase in the permeability of the surface

membrane to sodium ions, followed by a relatively prolonged increase in the permeability to potassium ions. The increased sodium conductance is responsible for the rising phase of the action potential and for the reversal of the membrane potential (Hodgkin, 1951). Interpreted in terms of the ionic hypothesis, the aforementioned results suggest that the sodium current in the active membrane is reduced in the drug treated muscles. Thus the suppression of the excitation and the amplitude of the action potential by the drugs tested appear to be related to a depression of the sodium ion permeability of the active membrane of the muscle fibre.

Treatment of the muscles with chlorpromazine, promethazine, morphine, meperidine, gamma-butyrolactone and low concentrations of gamma-hydroxybutyrate (5 and 10 x 10^{-3} g/ml) did not produce any alteration in the current-voltage relation curves. These observations suggest that the passive membrane conductance remains unaltered after the application of these drugs to the skeletal muscle. Inoue and Frank (1962) have reported similar effects with procaine on frog sartorius muscle fibres. In contrast, promethazine (5 x 10^{-6} g/ml), diphenhydramine, scopolamine, and high concentrations of gamma-hydroxybutyrate (15 x 10^{-3} g/ml) produced a counterclockwise rotation of the curves. Since the application of these drugs also increased the membrane time constant, the change in the current-voltage curves must have resulted from an increase in the effective membrane resistance between the inside and the outside of the fibre at the point of stimulation (Hodgkin and Rushton, 1946; Fatt and Katz, 1951).

Measurements obtained in the present experiments showed that promethazine (5×10^{-6} g/ml), diphenhydramine, scopolamine, and higher concentrations of gamma-hydroxybutyrate caused an increase of the effective membrane resistance. These observations provide further evidence for the statement that the counterclockwise rotation of the current-voltage curves must be as a consequence of the increased effective resistance of the membrane. Treatment with ether has also been shown to cause an increase in the membrane time constant, and the effective membrane resistance of the skeletal muscle (Inoue and Frank, 1965).

Studies of the current-voltage relation revealed two additional changes common to all the drugs and which also indicated an action on the sodium conductivity. These changes consisted of: (1) the suppression of the 'local response', and (2) the ability to produce larger depolarizations without the production of the propagated action potentials. There now exists considerable body of evidence to suggest that the 'local response' and the rising phase of the action potential are produced by a selective increase in sodium conductance of the fibre membrane (Hodgkin, 1951; Shanes, 1958). The observed depression of the 'local response' and the increase in the threshold depolarization both suggest that the drugs reported in the present study depress electrical excitability by interfering with the specific increase in sodium conductance accompanying the excitation of the fibres. It seems likely that the increase in the threshold value of the membrane depolarization for action potential production by all these drugs is

responsible for the observed increase in the threshold current. The observed increase in the threshold depolarization will also account for the slight decrease of the critical level of the membrane potential. The results obtained with procaine (Inoue and Frank, 1962) and ether (Inoue and Frank, 1965) also indicated an increase of the threshold depolarization and a suppression of the 'local response' in the sartorius muscle of the frog.

It was mentioned before (p. 15) that according to the ionic theory of membrane excitability the rate of rise and the overshoot of the action potential depend upon the sodium permeability, and the concentration gradient of sodium ions in the external medium (Hodgkin and Katz, 1949; Nastuk and Hodgkin, 1950). Electrophysiological studies have demonstrated that the anesthetics and the sodium ions behave as competitive antagonists (Crescitelli, 1952; Condouris, 1961; Inoue and Frank, 1962, 1965, 1967; Inoue et al., 1967; Shakalis and Condouris, 1967). The competitive antagonism hypothesis predicts that in a sodium-deficient medium the depressant action of the anesthetics would be intensified, whereas such an effect should be antagonized by raising the concentration of sodium ions in the bathing medium. In the present investigation it has been shown that addition of excess sodium (171 mM) into the extracellular medium antagonized the depressant action of all the aforementioned drugs on the rate of rise and on the overshoot of the action potential (used as a measure for sodium inward current). These findings not only lend a further support to the mechanism of action (i.e. sodium depressant effect) proposed here for the drugs used in this study, but also

substantiate the proposals made previously. Shanes (1958, 1963) has reviewed many studies that demonstrate a summation of the effects of local anesthetics and the treatments which decrease sodium currents, e.g. low external sodium, and depolarization by elevated external potassium or by applied electrical currents. He also quotes the experiments which indicate an antagonism between local anesthetics and treatments which enhance sodium currents, e.g. high external sodium, veratrine, and hyperpolarization by applied electrical currents.

There are several observers who support the view that a number of local and general anesthetics and many other drugs having general central nervous system depressant effects all block excitability in the active membrane by inhibiting the specific increase in membrane sodium conductivity. Examples of such studies have already been described in Chapter II. The significant point, of course, is to determine the precise nature of this sodium current reduction by anesthetics and other drugs producing narcosis, since this information may be of value in elucidating the mechanism operating at the molecular level. At present we can only speculate as to the nature of the interference, but sodium-potassium theory of the nerve impulse offers several interesting possibilities. Some of the speculations which have been made regarding the interfering mechanism of the anesthetics in the membrane (.e.g. closing or clogging of the sodium channels by the anesthetic agents, or the inactivation of the "sodium carrying system"), have been mentioned before (p. 32).

Hodgkin and Huxley (1952) have suggested that the sodium ions cross the cell membrane with the aid of a hypothetical "sodium carrying system" rather than by simple diffusion. They also showed that the rate of change in the sodium conductance is a function of the membrane potential, and the magnitude of the sodium current depends upon the activation of the "sodium carrying system" and the sodium concentration gradient across the membrane. It has been postulated that the anesthetic agents cause a reduction in the availability of the "sodium carrying system" and thus decrease the conductance of sodium in the active cell membrane (Weidmann, 1955; Thesleff, 1956). In a very recent article Frank (1970) has also made a parallel suggestion regarding the inhibition of the "sodium carrying system" by the local and general anesthetics as well as other central depressants. That the conduction block produced by certain local and general anesthetic agents can be counteracted by passing hyperpolarizing currents through the resting membrane (Weidmann, 1955; Inoue and Frank, 1965), has led to the belief that hyperpolarization of the membrane brings about the activation of the "sodium carrying system" which is rendered inactivated under the influence of the anesthetic drugs. In spite of the fact that practically nothing is known about the nature of the mechanisms which transport sodium and potassium across the membrane during activity, nonetheless inhibition of the "sodium carrying system" offers a working basis for studies on conduction block in nerve and muscle. Although the present experiments provide no evidence for such a mechanism of action (the "sodium carrying system"), but it seems

probable that this mode of action might also be involved for the drugs reported in this study.

In concluding it may be stated that a great variety of heterogenous group of centrally acting drugs currently classified according to their therapeutic use as sedatives, tranquilizers, analgesics and antihistaminics have one important property in common, that is, they all produce an anesthetic-like effect when applied to the isolated sartorius muscle of the frog. The results obtained suggest that the excitability depression produced by the drugs used in this study is due to a specific diminution of the sodium conductivity in the electrically excited fibre membrane. Similar results have been found using the frog's skeletal muscle for pentobarbital, tribromoethanol, paraldehyde, chloralose, chloral hydrate and urethane (Thesleff, 1956); chloroform, urethane and ether (Yamaguchi, 1961); procaine (Inoue and Frank, 1962); ether (Inoue and Frank, 1965); ethyl alcohol (Inoue and Frank, 1967); and hexafluordiethyl ether (Inoue *et al.*, 1967). The same mechanism of action has been proposed for procaine-induced conduction block in the squid giant axon (Taylor, 1959; Shanes *et al.*, 1959); and after applying tetrodotoxin, xylocaine and prochlorperazine to the large fibres of the sciatic nerve of the frog (Hille, 1966). Also the oral application of procaine (Frank and Sanders, 1963), and tetrodotoxin (Frank and Pinsky, 1966) has been shown to suppress the excitability of the isolated cerebral cortical slabs of the cat *in situ* by a similar mechanism of action. Thus there is a large body of evidence from many laboratories working on a variety

of tissues with a variety of techniques, showing that all general anesthetics studied and many other central depressant drugs all block excitability by suppression of the increase in sodium conductivity which normally follows an adequate stimulus.

The legitimate question that can be asked is whether the changes observed in the electrical properties of the skeletal muscle membrane in vitro, and the mechanism proposed, can be extrapolated to the known in vivo central depressant actions of the drugs used in the present series of experiments. Several years ago Thesleff (1956) suggested that the drugs capable of producing general central depression acted by a single fundamental mechanism of action at the cellular level both in the central nervous system and on peripheral excitable tissues. He further demonstrated that there was a positive correlation between the concentrations of the anesthetics (on a mg/ml basis) required to suppress sodium conductance in the isolated muscle and the doses required to produce central nervous system depression (on a mg/g body weight basis) in the intact frog. Subsequent studies showed that both local and general anesthetics as well as other central depressant drugs had similar effects in intact white mice and on isolated slabs of cerebral cortex (Frank and Sanders, 1963; Jhamandas, 1969). Hodgkin (1951) and Eccles (1953) have postulated that the excitation and impulse transmission in the central nervous system are caused by the same selective ionic conductances as has been observed in the nerve fibre (Hodgkin and Katz (1949); the skeletal muscle (Nastuk and Hodgkin, 1950); the heart muscle (Weidmann, 1955); and in the moto-

neurones (Coombs et al., 1955). The effects of a variety of central depressant agents have also been examined on all these tissues, and the results support the general opinion that the depression of excitability is due to a selective decrease in the active membrane permeability to sodium ions, the resting membrane potential however remaining essentially unaltered. In view of all these observations, it is highly probable that the hypnosis or the general depression of the central nervous system produced by all the drugs used in this investigation may involve a mechanism of action similar to that observed in the skeletal muscle fibres.

The following discussion pertains to the observations made with GABA and iso-osmolar concentrations of sucrose. The depressant effect of hypertonic solutions on the mechanical activity of the skeletal muscle has been recognized since long (Steggarda, 1927; Howarth, 1957), however, the fibres exposed to hypertonic solutions are electrically excitable and can conduct action potentials (Hodgkin and Horowicz, 1957). Fujino and co-workers (1961) report that if the frog's skeletal muscle is immersed in a Ringer's solution made hypertonic with sucrose, both the rate of rise and the rate of fall of the action potentials are decreased. The results obtained in this study with hypertonic sucrose solutions are therefore consistent with the observations made by Fujino and his colleagues.

Treatment with gamma-aminobutyric acid depressed the electrical excitability of the muscle fibres, as shown by the increase in the threshold current and the threshold depolarization necessary for

the production of action potentials. Results obtained by Sperelakis and Schneider (1968) indicate that the effective membrane resistance of the frog's sartorius muscle fibres is increased by hypertonic sucrose solution. Similar results were obtained in the present study with hypertonic Ringer's solution of sucrose and GABA. Increasing concentrations of GABA and iso-osmolar concentrations of sucrose also produced a progressive increase in the membrane time constant. The membrane resistance is inversely proportional to the conductance of the membrane which, in the resting state, is mainly due to the potassium permeability (Hodgkin, 1951). In the present experiments, hypertonic solutions of sucrose and GABA caused an increase in the effective membrane resistance as was shown by a counterclockwise rotation in the current-voltage relation curves. From these observations it may be inferred that the passive membrane conductance is decreased following the treatment with concentrated solutions of GABA and sucrose.

Unlike other drugs, addition of excess sodium into the extracellular medium did not antagonize the effect of GABA, but rather produced a further depression in the rate of rise and the overshoot of the action potential. These observations indicate a lack of anesthetic effect of GABA on the frog's sartorius muscle.

The results obtained in the present study revealed that all the effects of GABA on the electrical properties of the skeletal muscle membrane can easily be duplicated by using iso-osmolar concentrations of sucrose. These results therefore suggest that the GABA-induced depression of electrical excitability is most likely due to the hyper-

osmotic effect of the bathing solution on the fibre membrane. Although it is impossible to deduce from the present results a likely mode of action for the depressant effects of GABA as well as sucrose, it seems probable that the intimate mechanism of their action is different from the rest of the drugs reported in this study.

Jhamandas (1969) found that GABA had no central nervous system depressant effect when injected intraperitoneally into mice, but had similar effects to anesthetics and general central nervous system depressants on the cerebral cortex electrical activity when directly applied to the surface of the cortex. His results obviously mean, as previously shown (Curtis and Watkins, 1965), that GABA does not get past the blood-brain barrier. The results in the present study raise and provide proof for the even more interesting fact that when an anesthetic-like central nervous system depressant action for GABA can be shown, since GABA lacks an anesthetic-like effect on the skeletal muscle excitability it is probably producing its effects at synaptic sites. While the latter point is often assumed there is little proof for such a site of action.

CHAPTER VI

SUMMARY AND CONCLUSIONS

CHAPTER VI. SUMMARY AND CONCLUSIONS

1. The effects of a wide variety of central depressants (namely chlorpromazine, promethazine, diphenhydramine, scopolamine, meperidine, morphine, gamma-butyrolactone and gamma-hydroxybutyrate) on certain features of the active membrane related of sodium conductivity in skeletal muscle were examined. Studies with extracellular electrodes showed that increasing concentrations of these drugs caused a progressive depression of both the amplitude of the compound action potential and the excitability of the muscle fibres.

2. A slow and only partial recovery of the muscle fibres was noticed following the treatment with chlorpromazine, promethazine and diphenhydramine. However, with the rest of the drugs the depressed responses were reversed to the control level when the preparation was brought back to the normal Ringer's fluid.

3. Intracellular recordings showed that the resting membrane potential remained essentially unchanged in the drug treated muscles.

4. Diphenhydramine, promethazine (5×10^{-6} g/ml), scopolamine and gamma-hydroxybutyrate (15×10^{-3} g/ml) produced a counter-clockwise rotation of the current-voltage curves. Quantitative comparisons of the effective membrane resistance and the membrane time constant indicated that such a rotation was most likely due to the increased effective membrane resistance at the point of stimulation between the inside and the outside of the fibre membrane. The passive membrane conductance remained unaltered after the treatment with

chlorpromazine, promethazine, morphine, meperidine, gamma-butyrolactone and low concentrations of gamma-hydroxybutyrate (5 and 10×10^{-3} g/ml).

5. The drug-treated muscles showed a suppression of the 'local response', an increase in the threshold depolarization, and a slight decrease in the critical level of membrane potential. The threshold current required for the initiation of the action potential was increased by all the drugs used in this study.

6. The maximum rate of rise and the overshoot of the action potential were decreased by all the drugs. Both these effects were antagonized by increasing the extracellular sodium concentration.

7. The results obtained in this investigation suggest that a large variety of central depressant agents block electrical excitability of the sartorius muscle of the frog by suppressing the specific increase of sodium conductivity which normally follows depolarization of the fibre membrane. It is suggested that the hypnosis or the general depression of the central nervous system produced by all the forementioned drugs involves a similar mechanism of action to that observed in skeletal muscle fibres.

8. The lack of the anesthetic effect of gamma-aminobutyric acid on the frog's sartorius muscle was confirmed by using iso-osmolar concentrations of sucrose. It seems that the GABA-induced suppression of electrical excitability is due to the hyperosmotic effect of the bathing solutions on the fibre membrane. The observed decrease of sodium conductivity by GABA and sucrose in the active membrane may

involve some other mechanism of action than suggested for the rest of the drugs.

Bibliography

Bärlund, H. (1938). Influence of ethyl ether on the permeability of Chara cells. Protoplasma, 30: 70.

Bennett, A. L., Wagner, J. C. and McIntyre, A. R. (1942). The determination of local anesthetic potency by observation of nerve action potential. J. Pharmac. exp. Ther. 75: 125.

Bennett, A. L., Ware, F. Jr., Dunn, A. L. and McIntyre, A. R. (1954). The normal membrane resting potential of mammalian skeletal muscle measured in vivo. J. cell. comp. Physiol. 42: 343.

Bernstein, J. (1902). Untersuchungen zur thermodynamik der bioelektrischen Ströme. Pflügers Arch. ges. Physiol. 92: 521.

Bishop, G. H. (1932). Action of nerve depressants on potential. J. cell. comp. Physiol. 1: 177.

Boyle, P. J. and Conway, E. J. (1941). Potassium accumulation in muscle and associated changes. J. Physiol. 100: 1.

Brady, A. J. and Woodbury, J. W. (1960). The sodium-potassium hypothesis as the basis of electrical activity in frog ventricle. J. Physiol. 154: 385.

Brooks, S. C. (1947). Permeability and enzyme reactions. In Advances in Enzymology, vol. 7, pp.1-34, ed. Nord, F. F. New York: Interscience Publishers Inc.

Bülbring, E. (1954). Membrane potentials of smooth muscle fibres of the taenia coli of the guinea-pig. J. Physiol. 125: 302.

Bülbring, E. and Kuriyama, H. (1963). Effects of changes in the external sodium and calcium concentrations on spontaneous electrical activity in smooth muscle of guinea-pig taenia coli. J. Physiol. 166: 29.

Bunker, J. P. and Vandam, L. D. (1965). Effects of anesthesia on metabolism and cellular functions. Pharmac. Rev. 17: 183.

Caldwell, P. C. (1960). The phosphorus metabolism of squid axons and its relationship to the active transport of sodium. J. Physiol. 152: 545.

Coombs, J. S., Eccles, J. C. and Fatt, P. (1955). The electrical properties of the motoneurone membrane. J. Physiol. 130: 291.

Curtis, D. R. and Watkins, J. C. (1965). The pharmacology of amino acids related to gamma-aminobutyric acid. Pharmacol. Rev. 17: 347.

Camougis, G. and Takman, B. H. (1971). Nerve and nerve-muscle preparations (as applied to local anesthetics). In Methods in Pharmacology, vol. 1, pp.1-40, ed. Schwartz, A. New York: Appleton-Century - Crofts Educational Division, Meredith Corporation.

Carey, M. J. and Conway, E. J. (1954). Comparison of various media for immersing frog sartorii at room temperature, and evidence for the regional distribution of fibre Na^+. J. Physiol. 125: 232.

Clements, J. A. and Wilson, K. M. (1962). The affinity of narcotic agents for interfacial films. Proc. natn. Acad. Sci. U.S.A. 48: 1008.

Clements, J. A. and Wilson, K. M. (1963). Interactions of narcotic agents with spread lipid and protein films. In International Anesthesiology Clinics, vol. 1, No. 4, pp. 969-976, eds. Cullen, E. C. and Featherstone, R. M. Boston: Little, Brown and Company.

Condouris, G. A. (1961). A study of the mechanism of action of cocaine on amphibian peripheral nerve. J. Pharmac. exp. Ther. 131: 243.

Conway, E. J. (1946). Ionic permeability of skeletal muscle fibres. Nature, 157: 715.

Conway, E. J. (1947). Exchanges of K, Na and H ions between the cell and its environment. Ir. J. med. Sci., Series 6, No. 593, 654.

Conway, E. J. (1950). Calculation of the idiomolar value and its electrostatic equivalent in normal mammalian skeletal muscle. Ir. J. med. Sci., Series 6, No. 293, 216.

Conway, E. J. (1957). Nature and significance of concentration relations of potassium and sodium ions in skeletal muscle. Physiol. Rev. 37: 84.

Coraboeuf, E. and Otsuka, M. (1956). L'action des solutions hyposodique sur les potentiels cellulaire-de tissu cardiaque de Mammiferes. C.R. Acad. Sci., Paris, 243: part 1, 441.

Creese, R., D'Silva, J. L. and Northover, J. (1958). Serum factors which maintain muscle sodium at low levels. J. Physiol. 143: 22p.

Crescitelli, F. (1952). Modification in responses to sodium of nerve fibres treated with drugs. Am. J. Physiol. 169: 638.

Davson, H. and Danielli, J. F. (1952). The permeability of natural membranes. pp. 231-243. London: Cambridge Univ. Press.

Desmedt, J. E. (1953). Electrical activity and intracellular sodium concentration in frog muscle. J. Physiol. 121: 191.

Draper, M. H. and Weidmann, S. (1951). Cardiac resting and action potentials recorded with an intracellular electrode. J. Physiol. 115: 74.

Eccles, J. C. (1953). The neurophysiological basis of mind. 1st ed. p. 4. London: Oxford Univ. Press.

Ehrenpreis, S. (1964). Acetylcholine and nerve activity. Nature, 201: 887.

Fatt, P. and Katz, B. (1951). An analysis of the end-plate potential recorded with an intracellular electrode. J. Physiol. 115: 320.

Fatt, P. and Katz, B. (1953). The electrical properties of crustacean muscle fibres. J. Physiol. 120: 171.

Feinstein, M. B. (1964). Reaction of local anesthetics with phospholipids (a possible chemical basis of anesthesia). J. gen. Physiol. 48: 357.

Feinstein, M. B. and Paimre, M. (1966). Specific reaction of local anesthetics with phosphodiester groups. Biochim. biophys. Acta, 115: 33.

Ferroni, A. and Blanchi, D. (1965). Maximum rate of depolarization of single muscle fiber in normal and low sodium solutions. J. gen. Physiol. 49: 17.

Frank, G. B. (1957). The effects of trematode infection on the physiological activity of the frog. Can. J. Biochem. Physiol. 35: 347.

Frank, G. B. (1958). Effect of veratrine on muscle fibre membrane and on negative after-potential. J. Neurophysiol. 21: 263.

Frank, G. B. and Sanders, H. D. (1963). A proposed common mechanism of action for general and local anaesthetics in the central nervous system. Br . J. Pharmac. Chemother. 21: 1.

Frank, G. B. and Pinsky, C. (1966). Tetrodotoxin-induced central nervous system depression. Br. J. Pharmac. Chemother. 26: 435.

Frank, G. B. (1970). The effects of anaesthetic drugs based on alteration of membrane excitability. In Press.

Freygang, W. H. Jr., Goldstein, D. A., Hellam, D. C., and Peachey, L. D. (1964). The relation between the late after-potential and the size of the transverse tubular system of frog muscle. J. gen. Physiol. 48: 235.

Fujino, M., Yamaguchi, T. and Suzuki, K. (1961). 'Glycerol effect' and the mechanism linking excitation of the plasma membrane with contraction. Nature, 192: 1159.

Gershfeld, N. L. and Shanes, A. M. (1959). Antagonism of veratrine by calcium ions in monolayers of stearic acid. Science, 129: 1427.

Goldman, D. E. (1943). Potential, impedance and rectification in membranes. J. gen. Physiol. 27: 37.

Grundfest, H. (1961). Ionic transport across neural and non-neural membranes. In: Properties of membranes and diseases of nervous system, ed. Tower, D. B., Luse, S. A. and Grundfest, H. p.71. New York: Springer Publishing Co. Inc.

Hagiwara, S. and Watanabe, A. (1955). The effect of tetraethylammonium chloride on the muscle membrane examined with an intracellular electrode. J. Physiol. 129: 513.

Haglund, B. and Lovtrup, S. (1966). Anaesthesia and water permeation. Acta Biochim. Polo. 13: 395.

Hastings, A. B. and Eichelberger, L. (1937). The exchange of salt and water between muscle and blood. J. biol. Chem. 117: 73.

Hille, B. (1966). Common mode of action of three agents that decrease the transient change in sodium permeability in nerves. Nature, 210: 1220.

Höber, R. (1945). Physical chemistry of cells and tissues. pp.355-370. Philadelphia: The Blakiston Co.

Hodgkin, A. L. (1938). The subthreshold potentials in a crustacean nerve fibre. Proc. R. Soc. B., 126: 87.

Hodgkin, A. L., Huxley, A. F. and Katz, B. (1949). Ionic currents underlying activity in the giant axon of the squid. Archs Sci. physiol. 3: 129.

Hodgkin, A. L. and Katz, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol. 108: 37.

Hodgkin, A. L. (1951). The ionic basis of electrical activity in nerve and muscle. Biol. Rev. 26: 339.

Hodgkin, A. L. and Huxley, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. 117: 500.

Hodgkin, A. L. and Horowicz, P. (1957). The differential action of hypertonic solutions on the twitch and action potential of a muscle fibre. J. Physiol. 136: 17P.

Hodgkin, A. L. (1958). The Croonian Lecture: Ionic movements and electrical activity in giant nerve fibres. Proc. R. Soc. B. 148: 1.

Hodgkin, A. L. and Horowicz, P. (1959). Movements of Na and K in single muscle fibres. J. Physiol. 145: 405.

Hodgkin, A. L. (1964). The conduction of the nervous impulse. Liverpool: University Press.

Howarth, J. V. (1957). The effect of hypertonic solutions on the velocity of shortening of the frog's sartorius. J. Physiol. 137: 23P.

Huxley, A. F. and Stämpfli (1949). Evidence for saltatory conduction in peripheral myelinated nerve fibres. J. Physiol. 108: 315.

Huxley, A. F. and Stämpfli, R. (1951). Effect of potassium and sodium on resting and action potentials of single myelinated nerve fibres. J. Physiol. 112: 496.

Inoue, F. and Frank, G. B. (1962). Action of procaine on frog skeletal muscle. J. Pharmac. exp. Ther. 136: 190.

Inoue, F. and Frank, G. B. (1965). Action of ether on frog skeletal muscle. Can. J. Physiol. Pharmacol. 43: 751.

Inoue, F. and Frank, G. B. (1967). Effects of ethyl alcohol on excitability and on neuromuscular transmission in frog skeletal muscle. Br. J. Pharmac. Chemother. 30: 186.

Inoue, F., Pinsky, C. and Sanders, H. D. (1967). A comparison of the effects of hexafluordiethyl ether and pentylenetetrazol on the excitability of frog skeletal muscle fibres. Can. J. Physiol. Pharmacol. 45: 757.

Jacobs, M. H. and Parpart, A. K. (1937). The influence of certain alcohols on the permeability of the erythrocyte. Biol. Bull. 73: 380.

Jarvik, M. E. (1970). Drugs used in the treatment of psychiatric disorders. In: The pharmacological basis of therapeutics. 4th ed. pp.151-203. eds. Goodman, L. S. and Gilman, A. London: The MacMillan Company, Collier-MacMillan.

Jenerick, H. P. and Gerard, R. W. (1953). Membrane potential and threshold of single muscle fibres. J. cell. comp. Physiol. 42: 79.

Jhamandas, K. (1969). The anesthetic properties of drugs which act in the central nervous system. A doctoral degree thesis, University of Alberta, Edmonton, Alberta.

Katz, B. (1937). Experimental evidence for a non-conducted response of nerve to subthreshold stimulation. Proc. R. Soc. B. 124: 244.

Katz, B. (1966). Nerve, muscle and synapse. New York: McGraw-Hill Book Company.

Kernan, R. P. and Conway, E. J. (1955). Muscle fibre membrane potentials in relation to external potassium concentration. In: Abstracts of the international biochemical Congress, Brussels.

Kernan, R. P. (1960). Resting potentials in isolated frog sartorius fibres at low external potassium concentrations. Nature, 185: 471.

Kernan, R. P. (1963). The resting potential of isolated rat muscles measured in plasma. Nature, 200: 474.

Kernan, R. P. (1965). Cell, K. Washington: Butterworth's.

Liebe, H. (1948). Zur theorie der narkose. Untersuchungen über die Ionendurchlässigkeit der roten Blutkörperchen im Hinblich auf die Permeabilitatstheorie der Narkose. Arch. ges. Physiol.250: 295.

Lillie, R. S. (1923). Protoplasmic action and nervous action. pp.206-216. Chicago, Illinois: The University of Chicago Press.

Ling, G. and Gerard, R. W. (1949). The normal membrane potentials of frog sartorius fibres. J. cell. comp. Physiol. 34: 383.

Ling, G. and Woodbury, J. W. (1949). Effect of temperature on the membrane potential of frog muscle fibres. J. cell. comp. Physiol. 34: 407.

Lorente, de Nó, R. (1949). On the effect of certain quarternary ammonium ions upon frog nerve. J. cell. comp. Physiol. 33: (Suppl.) 1.

MaCallum, A. B. (1905). On the distribution of potassium in animal and vegetable cells. J. Physiol. 32: 95.

Moore, J. W. and Cole, K. S. (1960). Resting and action potentials of squid giant axon in vivo. J. gen. Physiol. 43: 961.

Nastuk, W. L. and Hodgkin, A. L. (1950). The electrical activity of the single muscle fibres. J. cell. comp. Physiol. 35: 39.

Osterhout, W. J. V. (1913). Effect of anesthetics on permeability. Science, 37: 111.

Overton, E. (1902). Beiträge zur allgemeinen muskel - und nervenphysiologie. Pflügers Arch. ges. Physiol. 92: 346.

Pauling, L. (1961). A molecular theory of general anesthesia. Science, 134: 15.

Pittinger, C. B. and Keasling, H. H. (1959). Theories of narcosis. Anesthesiology, 20: 204.

Quastel, J. H. (1962). Effects of anesthetics, depressants, and tranquilizers on brain metabolism. In: Neurochemistry; the chemistry of brain and nerve. 2nd ed. pp.790-812, eds. Elliot, K. A., Page, I. H. and Quastel, J. H. Springfield: Charles C. Thomas.

Rideal, E. K. (1945). Surface chemistry in relation to biology. Endeavour, 4: 83.

Ritchie, J. M. and Greengard, P. (1961). On the active structure of local anesthetics. J. Pharmac. exp. Ther. 133: 241.

Ritchie, J. M., Cohen, P. J. and Dripps, R. D. (1965). Local anesthetics. In: The pharmacological basis of therapeutics. 3rd ed., Chapter 20, p.374, eds. Goodman, L. S. and Gilman, A. New York: The MacMillan Company.

Schoepfle, G. M. and Grant, J. M. (1954). Relation between electrotonic potential and membrane resistivity in frog nerves. Am. J. Physiol. 177: 187.

Shakalis, A. and Condouris, G. A. (1967). Local anesthetic action of cocaine on nerves from adrenalectomized frogs. Arch. int. Pharmacodyn. Thér. 166: 93.

Shanes, A. M. (1958). Electrochemical aspects of physiological and pharmacological action in excitable cells. Pharmac. Rev. 10: 165.

Shanes, A. M., Freygang, W. H., Grundfest, H. and Amatniek, E. (1959). Anesthetic and calcium action in the voltage clamped squid giant axon. J. gen. Physiol. 42: 793.

Shanes, A. M. and Gershfeld, N. L. (1960). Interactions of veratrum alkaloids, procaine, and calcium with monolayers of stearic acid and their implications for pharmacological action. J. gen. Physiol. 44: 345.

Shanes, A. M. (1962). Electrolytes and the function and pharmacology of nerve. In: Neurochemistry. 2nd ed. pp. 558-577, eds. Elliot, K. A. C., Page, R. and Quastel, J. H. Springfield, Illinois: Charles C. Thomas.

Skou, J. C. (1958). Relation between the ability of various compounds to block nervous conduction and their penetration into a monomolecular layer of nerve-tissue lipoids. Biochim. Biophys. Acta, 30: 625.

Skou, J. C. (1961). The effect of drugs on cell membranes with special reference to local anesthetics. J. Pharm. Pharmac. 13: 204.

Sperelakis, N. and Schneider, M. F. (1968). Membrane ion conductances of frog sartorius fibers as a function of tonicity. Am. J. Physiol. 215: 723.

Steggarda, F. R. (1957). Effects of water content on muscular efficiency. Proc. Soc. exp. Biol. Med. 24: 915.

Straub, R. (1956a). Effects of local anesthetics on resting potential of myelinated nerve fibres. Experientia, 12: 182.

Straub, R. (1956b). Der Einfluss von Lokalanesthetika auf Ionenbedingte Ruhepotentialänderungen von Markhaltigen Nervenfasern des Frosches. Arch. int. Pharmacodyn. Thér. 107: 414.

Tasaki, I., Polley, E. H. and Orrego, F. (1954). Action potential from individual elements in cat geniculate and striate cortex. J. Neurophysiol. 17: 454.

Taylor, R. E. (1959). Effect of procaine on electrical properties of squid axon membrane. Amer. J. Physiol. 196: 1071.

Thesleff, S. (1956). The effect of anesthetic agents on skeletal muscle membrane. Acta physiol. scand. 37: 335.

Toman, J. E. P. (1962). Neurotropic drugs. In: Neurochemistry, 2nd ed. pp. 728-765. eds. Elliot, K. A. C., Page, R. and Quastel, J. H. Springfield, Illinois: Charles C. Thomas.

Ussing, H. H. (1949). Transport of ions across cellular membranes. Physiol. Rev. 29: 127.

Verworn, M. (1913). Irritability: A physiological analysis of the general effect of stimuli in living substance. Chapter 9. New Haven: Yale University Press.

Watson, P. J. (1960). The mode of action of local anesthetics. J. Pharm. Pharmac. 12: 257.

Weidmann, S. (1955). Effects of calcium ions and local anaesthetics on electrical properties of purkinje fibres. J. Physiol. 129: 568.

Winterstein, H. (1915). Beiträge zur Kenntnis der Narkose. III. Mitteilung. Narkose und Erstickung. Biochem. Z. 70: 130.

Woodbury, J. W. (1965). Biophysics of the cell membrane. In: Physiology and Biophysics. Eds. Ruch, T. C. and Patton, H. D. 19th ed. p.5. Philadelphia: W. B. Saunders Company.

Yamaguchi, T. (1961). Electrophysiological studies on the mechanism of effects of anaesthetics on the isolated frog muscle fibre. J. Fac. Sci., Hokkaido Univ., Ser. VI, Zool., 14: 522.

Yamaguchi, T. and Okumura, H. (1963). Effects of anaesthetics on the electrical properties of the cell membrane of the frog muscle fibre. Annot. Zool. Jap., 36: 109.