

Acetylcholine and Calcium on Membrane Permeability and Contraction of Intestinal Smooth Muscle

LEON HURWITZ, STANLEY VON HAGEN, and PAUL D. JOINER

From the Department of Pharmacology, School of Medicine, Vanderbilt University,
Nashville, Tennessee

ABSTRACT Acetylcholine elicited a sustained contraction and an increase in potassium efflux in longitudinal muscle isolated from the guinea pig ileum. Stepwise increases in the calcium concentration of the bathing medium from 0.06 to 36 mM generally reduced the increase in potassium efflux, but had a complex effect on the mechanical response. Contractions produced by high levels of acetylcholine became progressively larger or remained at a high magnitude as the calcium concentration was increased. Contractions produced by low levels of acetylcholine also improved initially, but were depressed again by the highest concentration of calcium introduced. Ethanol, in the appropriate concentration, inhibited completely the acetylcholine-induced contraction without reducing the increase in potassium efflux. Calcium reversed this effect. Both extracellular calcium and ethanol depressed the large, transient increase in muscle tone developed by fibers that were preincubated in a high calcium medium and then exposed to a calcium-free medium. These findings suggested that extracellular calcium ions react with two different sites in the membrane, a stabilizing site and a storage site. A muscle contraction is activated by calcium ions which diffuse from the storage site to the myoplasm. Calcium ions reacting with the stabilizing site impede this diffusion process. Part of the stimulatory effect of acetylcholine is derived from its capacity to counteract the action of calcium at the stabilizing site.

There is presently little doubt that cholinergic agents increase membrane permeability of intestinal smooth muscle fibers to inorganic ions. This conclusion is based on evidence obtained both from electrophysiological studies (2, 3) and studies of transmembrane ion movements (9, 17, 26). The significant question that remains to be answered is how drug-induced changes in

membrane permeability and polarization are associated with changes in the mechanical activity of the muscle fiber. According to classical theory an excitatory drug, by increasing the permeability of the fiber membrane, facilitates the development of action potentials. The action potential then initiates a series of reactions which lead to an increase in smooth muscle tension. This concept was shown to be inadequate by the demonstration that excitatory drugs could evoke an increase in tension from depolarized smooth muscle without producing any change in membrane potential (11, 12). Subsequently, various suggestions have been offered to explain the manner by which excitatory drugs stimulate smooth muscle fibers to contract. These include the concepts that (*a*) the drug directly increases the permeability of the membrane to calcium ions (8); (*b*) the drug causes the release of calcium ions from a membrane store (10), and (*c*) membrane depolarization initiates the release of calcium primarily from one membrane store whereas, the excitatory agent releases it from another, or perhaps from more than one store (5, 14, 24).

It is apparent, therefore, that the relationship between activity in the plasma membrane and activity of contractile elements in smooth muscle is not fully understood. In this study the question was investigated by altering the extracellular calcium ion concentration in special ways and by introducing an inhibitory agent into the bathing solution. We then determined how these experimental procedures affected the changes in transmembrane potassium movements and the changes in smooth muscle tone produced by acetylcholine and other stimuli. The work was performed on the longitudinal smooth muscle isolated from guinea pig ileum. From the experimental results obtained it was possible to devise a model which illustrates proposed interactions between acetylcholine and calcium ions in the fiber membrane and indicates how these interactions relate to the mechanical activity of this smooth muscle preparation.

METHODS

The longitudinal smooth muscle employed in this study was isolated from guinea pig ileum by the method described by Weiss et al. (26). A segment approximately 3 cm long was suspended in a muscle bath which contained 10 ml of a physiological salt solution. The temperature of the bath was maintained at 33–34°C. The tension on the muscle was approximately 0.35 g.

The physiological salt solution had the following composition: NaCl, 125 mM; KCl, 2.7 mM; CaCl₂, 1.8 mM; glucose, 11 mM and Tris [tris(hydroxymethyl) amino-methane, sigma-trizma base] buffer, 23.8 mM. The solution was adjusted to pH 7.5 with 6 N HCl and was saturated with 100 per cent oxygen.

The drugs and agents used in this investigation were usually added directly to the bathing fluid from concentrated stock solutions. The compounds used included acetylcholine chloride, ethanol, and calcium chloride. If by adding the solutions

which contained these compounds the bathing medium would be diluted more than 5 per cent, the bathing medium was replaced instead by one already containing the appropriate concentration of the agent and made up to the final correct volume.

Isotonic contractions of the muscle were recorded on a standard kymograph.

The method for measuring potassium efflux was essentially that described by Hurwitz et al. (16). A tissue was made radioactive by incubating it for 2-3 hr in a solution containing ^{42}K . It was then resuspended in 10 ml of nonradioactive medium. In each experiment the solution bathing the tissue was removed at 5-min intervals and replaced by an equal quantity of fresh nonradioactive solution. The bathing

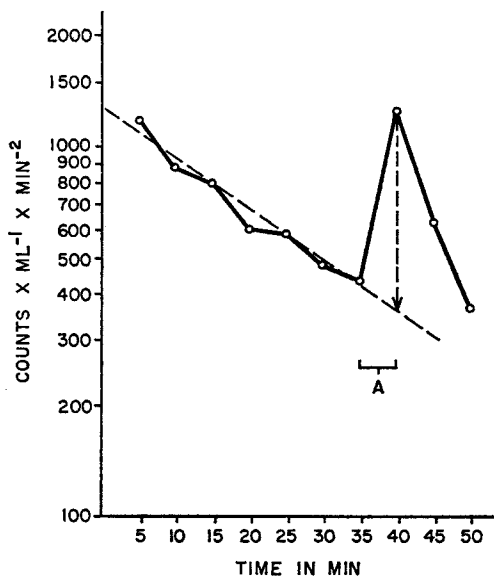


FIGURE 1 Potassium efflux from isolated longitudinal smooth muscle. Acetylcholine ($1.4 \times 10^{-6} \text{ M}$) was introduced into the muscle bath for the 5-min period indicated by the braces.

solution removed during the first 15 min was discarded to allow the system to reach a state of steady efflux. The radioactivity of each subsequent 5-min collection was measured. In this way the rate of escape of ^{42}K from the ileal muscle could be determined.

Measurements of radioactivity were made on 2 ml aliquots of each 5-min sample. The fluid was pipetted into planchets and evaporated to dryness under a heat lamp. Counts were obtained in a counting unit with an end-window Geiger tube and an appropriate scaling circuit. Corrections were made for the disintegration of ^{42}K during the counting procedure.

Acetylcholine elicits a sustained contraction and an increase in potassium efflux in the isolated longitudinal muscle. The method for estimating the increase in potassium efflux is indicated in Fig. 1. The average rate of the outward movement of ^{42}K for a 5-min period was experimentally determined for a number of successive 5-min

periods. The data denoting the rates of outward movement of ^{42}K from the tissue (given as counts $\times \text{ml}^{-1} \times \text{min}^{-2}$) were plotted on a logarithmic scale at points on the graph which were linearly spaced and represent the ends of successive 5-min intervals. The values of the ^{42}K efflux at all 5-min intervals in which the muscle was in an unexcited state were connected by the best straight line as shown by the broken line in Fig. 1. This line is considered to be the normal regression line for the decline of ^{42}K efflux with time in an unexcited muscle. The rate constant for the efflux of potassium from unexcited muscle may be calculated from the slope of this normal regression line. The ^{42}K efflux estimated from an extension of the normal regression line and the ^{42}K efflux obtained experimentally in the presence of drugs were used to calculate the average percentage change in potassium efflux in a 5-min period resulting from the introduction of drugs. This calculated percentage change in the rate of outflow of potassium approximates the average percentage change in the rate constant for the efflux of potassium. The data obtained on potassium movements in stimulated and unstimulated muscle will be presented in the figures below as the rate constants for the efflux of potassium ion.

In experiments in which acetylcholine was the excitatory agent, the magnitude of an induced isotonic contraction was considered to be the vertical distance between points representing resting tone and the highest level of tone reached by the muscle during the 5-min interval that cholinergic agent was present in the bath. One experimental and one control or reference response was obtained in each muscle preparation tested. Variations in magnitudes of contraction noted under different experimental conditions are presented as per cent of the control or reference response.

The data on potassium fluxes and smooth muscle contractions shown in Figs. 2-5 below are mean values of a minimum of 10 experimental determinations obtained in different tissue preparations. Wherever a definite statement is made in the text about a difference between one mean value or one group of mean values and another, it indicates that the difference is significant at the 5% level.

In Fig. 6 the records of two individual experiments are presented. Each was selected as a typical representation of the manner in which the muscle reacted in eight experiments performed on different tissues.

RESULTS

To provide some indication of the interrelationship between acetylcholine-induced changes in membrane activity and mechanical state of the muscle the effects of calcium ion on smooth muscle responses were investigated. Calcium was selected for investigation because it acts as a depressant of membrane excitability (13, 22, 23, 25), but enhances the operation of the contractile machinery (1, 7, 21). Thus, by varying the calcium ion concentration in the external medium it is possible to ascertain the ultimate effect on mechanical response which results from progressively increasing the muscle's capacity to contract while simultaneously decreasing the change in membrane permeability that can be induced by a given stimulus.

As an index of alterations in membrane permeability the unidirectional

efflux of potassium ions from the muscle fibers was measured. Theoretically, an increase in membrane permeability to potassium ion and/or an increase in membrane permeability to ions which cause membrane depolarization will be reflected by an increase in the rate constant for the passive outflow of potassium from the cell (4, 15, 22).

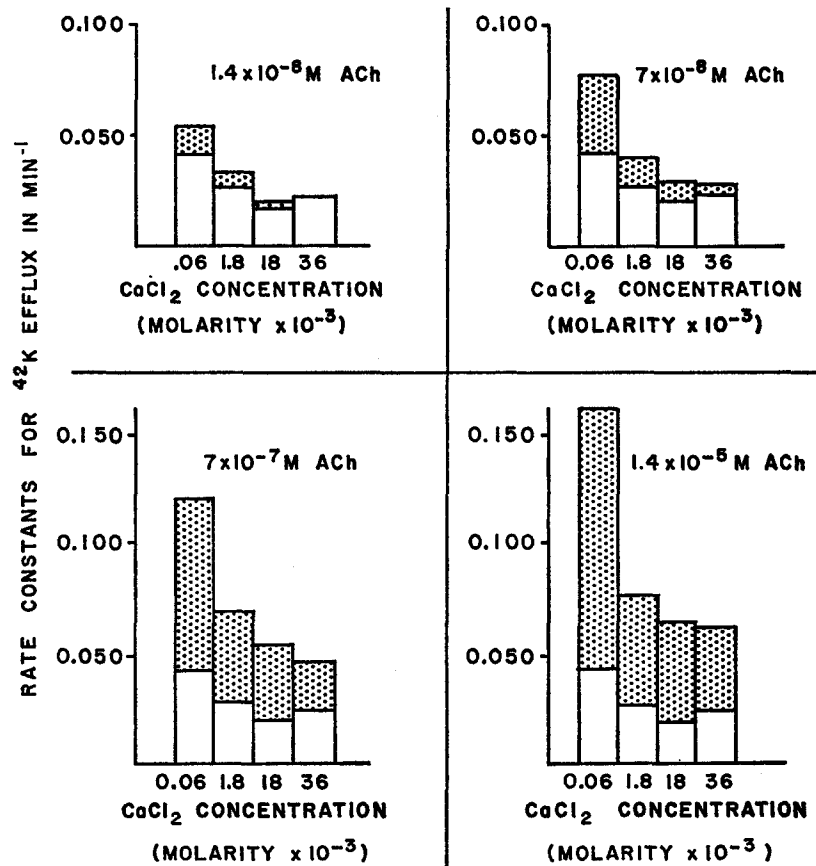


FIGURE 2 Mean values of the rate constants for the efflux of potassium ion from isolated longitudinal muscle. Unshaded areas indicate the rate constants in unstimulated muscle. Shaded areas show the increase in rate constants in acetylcholine-stimulated muscle.

The experimental data presented in Fig. 2 show how different concentrations of calcium ion in the bathing fluid affect the rate constant for the outward movement of potassium from the longitudinal muscle in the presence of various concentrations of acetylcholine. The experiments were performed on tissues that had first been incubated for 2 hr in bathing solutions that contained the desired concentrations of calcium ion. During the experiment

acetylcholine was added to the bathing fluid for a 5-min period. It produced an increase in the rate of outflow of potassium ions from the muscle fibers. The rate constants for the potassium efflux varied with the concentration of acetylcholine introduced. The higher the level of the cholinergic agent employed, the greater was the resulting rate constant. Variations in the extracellular calcium ion concentration modified the rate constants for potassium efflux irrespective of the level of acetylcholine present. Increasing the calcium ion concentration from 0.06 to 1.8 mM caused a striking reduction in the rate constant for the efflux of potassium. Increasing the calcium concentration another order of magnitude from 1.8 to 18 mM caused an additional small diminution in the rate constant. When the concentration of calcium was raised to 36 mM, the mean values of the rate constants seemed to be lowered somewhat more, but they were not significantly different from the mean values observed in the presence of 18 mM calcium. Thus, progressive increases in the calcium concentration produced either increasingly greater reductions in the rate constant for potassium efflux from the muscle or maintained a low order of activity of this membrane process.

The effect of increasing concentrations of the divalent ion on the muscle contraction elicited by acetylcholine followed a more complex pattern. The experimental results are shown in Fig. 3. When the calcium concentration in the medium was elevated from 0.06 to 1.8 mM, the mechanical response of the muscle was consistently enhanced. A further increase in the calcium concentration to 18 mM did not produce any additional significant change in the magnitudes of contraction produced by the various concentrations of acetylcholine. The introduction of 36 mM calcium ion into the medium maintained or enhanced slightly the contractile response to high levels of acetylcholine but had a decided inhibitory effect on the muscle contractions produced by the two lower levels of acetylcholine. Inhibition of the mechanical response did not occur if instead of 36 mM calcium, the osmotically equivalent substances, 1.8 mM calcium and 58.5 mM Tris ion, were added to the bathing solution.

These data show that calcium ion, within the concentration range studied, consistently depresses the transmembrane movement of potassium ion, but exhibits both stimulatory and inhibitory effects on the mechanical response of the muscle. The poor contractile response observed in the presence of low levels of calcium may be attributed to an inadequate supply of calcium necessary to fully activate the contractile machinery. The poor contractile response observed in the presence of high levels of calcium and low levels of acetylcholine may indicate that the divalent ion increases membrane resistance to its own inward diffusion. The latter suggestion is supported by the findings that calcium ion is an inhibitor of transmembrane potassium movement and that it does not depress increases in smooth muscle tone induced by high concentrations of acetylcholine.

It was shown in previous studies that the addition of an appropriate concentration of ethanol to the bathing solution antagonized some of the actions of calcium ion on the longitudinal smooth muscle (19, 27). Ethanol depressed the contraction and reduced the increase in potassium efflux elicited by acetylcholine. Both these effects could be counteracted by elevating the calcium concentration of the medium. In this report, the results of a more

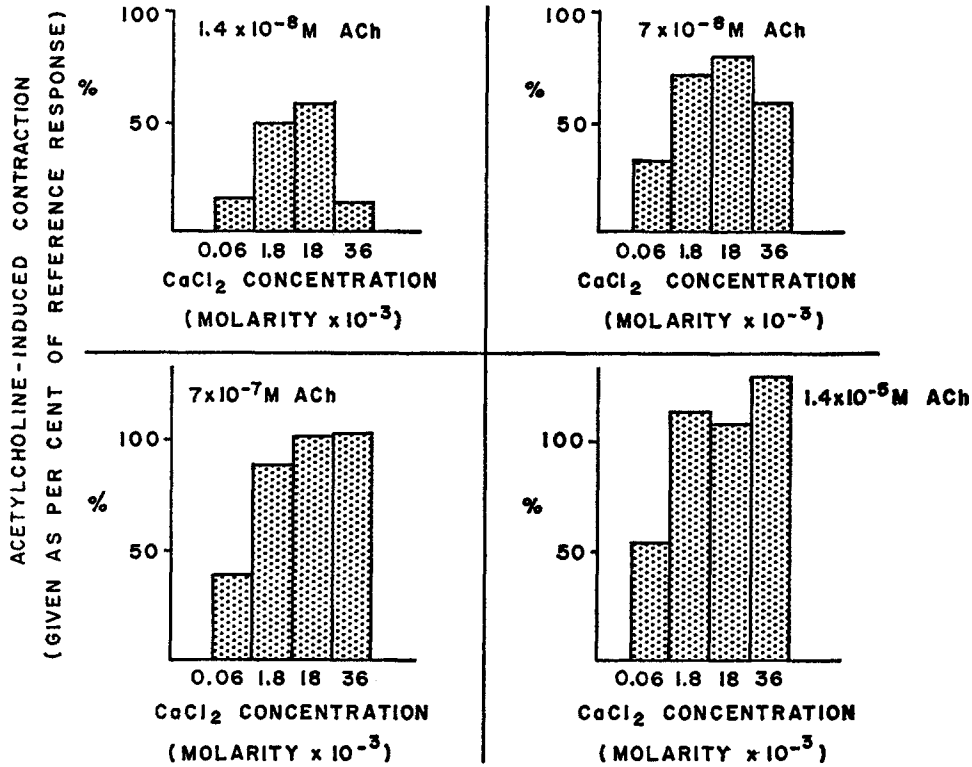


FIGURE 3 Acetylcholine-induced contractions in isolated longitudinal muscle. Each bar indicates the mean value for the increase in smooth muscle tone given as per cent of a reference response. The reference response was the contraction produced by 7×10^{-6} M acetylcholine in a muscle exposed to a medium which contained 1.8×10^{-3} M CaCl_2 .

extensive investigation are presented. We determined the influence of varying concentrations of ethanol on the magnitude of contraction and the increase in potassium efflux produced by 1.4×10^{-5} M acetylcholine in the longitudinal fibers. The manner in which different concentrations of calcium ion modified the actions of ethanol was also examined. In each experiment the muscle preparation was incubated for 2 or more hr in a bathing solution that contained the desired concentration of calcium ion. Increases in muscle tone and potassium efflux in the acetylcholine-stimulated tissue were then measured

by the procedure described in the Methods section. When ethanol was included in the experiment, it was introduced into the bathing fluid 1 min prior to the addition of acetylcholine and remained in the solution for the 5-min period during which the acetylcholine was present in the bath.

As is evident in Fig. 4, progressive elevations in the ethanol concentration produced increasingly greater inhibitions of the contraction produced by

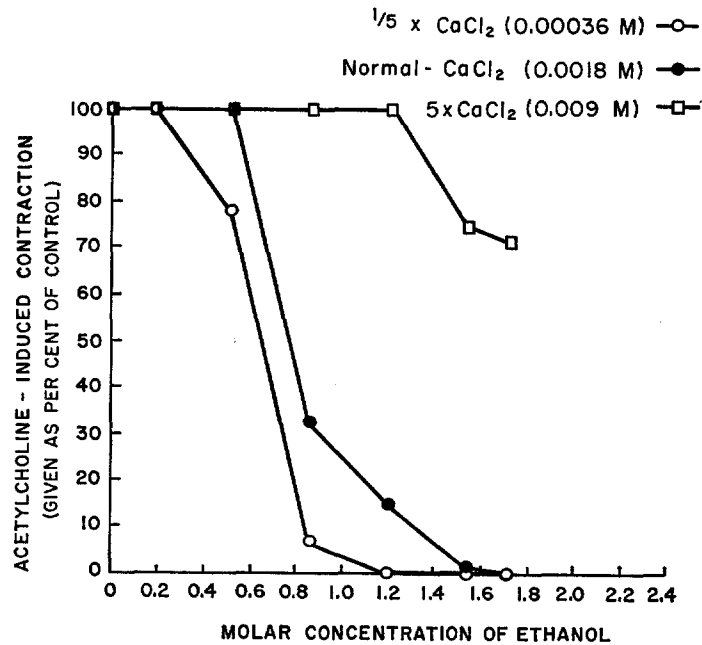


FIGURE 4 Isotonic contractions produced by 1.4×10^{-5} M acetylcholine in the presence of different concentrations of ethanol and CaCl_2 . Each point represents the mean value of the contraction given as per cent of control response. The control response was the contraction produced by 1.4×10^{-5} M acetylcholine in the absence of ethanol. The experimental and control responses that were compared were obtained in the presence of equal concentrations of CaCl_2 .

acetylcholine until a state of complete depression of mechanical activity was reached. The degree of inhibition produced by any given level of ethanol was dependent upon the concentration of calcium ion in the medium. Within the range of concentrations tested (0.36–9.0 mM), the higher the level of calcium present, the smaller was the inhibitory effect of ethanol. Thus, both ethanol and calcium deprivation modify acetylcholine-induced increases in smooth muscle tone in a similar fashion.

The effect of ethanol on the efflux of potassium ion from an acetylcholine-stimulated tissue immersed in a normal physiological bathing solution followed a more complex pattern. It is presented in Fig. 5 by a biphasic curve. Step-

wise increases in ethanol concentration from 0.17 to 0.86 M caused a progressively greater augmentation of the potassium efflux, increases in ethanol concentration from 0.86 to 1.54 M produced a progressively smaller augmentation of potassium efflux, and 1.72 M ethanol inhibited the rate of potassium outflow

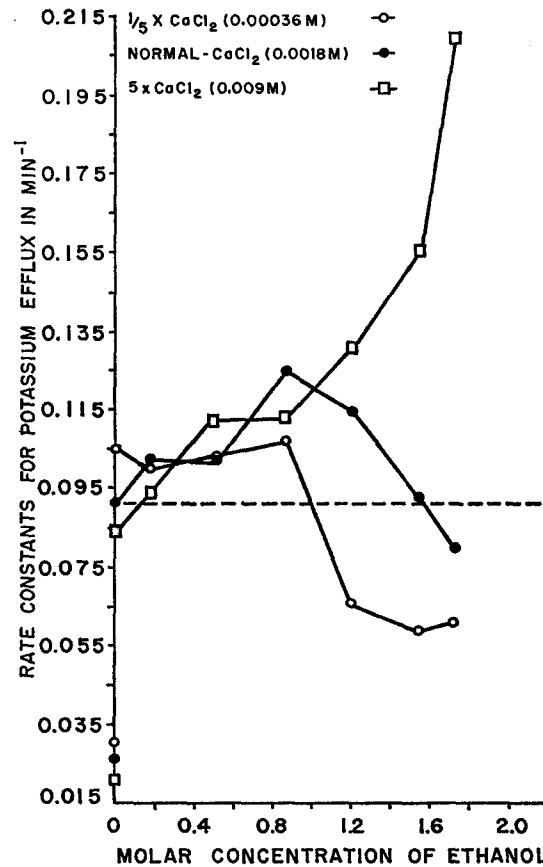


FIGURE 5 Mean values of the rate constants for the efflux of potassium ion induced by 1.4×10^{-5} M acetylcholine in the presence of different concentrations of ethanol and CaCl_2 . The three points shown in the lower left corner of the graph represent the rate constants in the unstimulated muscle. All other points represent the rate constants in acetylcholine-stimulated muscle. The dashed line indicates the magnitude of the rate constant for the potassium efflux from muscle stimulated by 1.4×10^{-5} M acetylcholine, in the presence of 1.8×10^{-3} M CaCl_2 , and in the absence of ethanol.

evoked by acetylcholine. These effects cannot be attributed to changes in the base line level of potassium efflux because concentrations of ethanol up to 0.86 M had no effect on this membrane process in unstimulated muscles and higher concentrations elevated it somewhat. Increasing the calcium ion concentration of the medium accentuated the stimulatory effect of ethanol on

potassium efflux; lowering the calcium ion concentration accentuated the inhibitory effect of ethanol. These observations are also represented in Fig. 5.

The way in which increasing concentrations of ethanol alter potassium movement in the acetylcholine-stimulated muscle resembles the pattern one might expect from a progressive diminution in the level of extracellular calcium ion. A reduction in the calcium ion concentration of the bathing medium accelerates the outward movement of potassium. If, however, the level of calcium is drastically reduced (with the use of EDTA), there appears to be some inhibition of this membrane process (27). Thus, ethanol and calcium deprivation produce similar effects on the outward flow of potassium which occurs in the presence of acetylcholine.

There are two aspects of ethanol's action that are worthy of emphasis. One is the inhibition of the mechanical response that is produced while the efflux of potassium remains unaffected or is even augmented; the other is the antagonism that exists between the action of ethanol and the action of calcium ion on the mechanical response of the muscle. These findings introduce the possibility that ethanol somehow depletes or renders ineffective a cellular supply of calcium which activates the contractile machinery during an acetylcholine-induced contraction. It is highly improbable that ethanol inhibits the smooth muscle contraction by complexing with calcium ions in the external solution. Nor does the drug seem to impede the movement of inorganic ions across the cell membrane.

The suggestion that both ethanol and extracellular calcium ions can influence actions of calcium ions that originate from an internal store in the muscle fiber was tested by another procedure. The longitudinal muscle was incubated for approximately 2 hr in a bathing solution that contained 36 mM calcium chloride. Following this prolonged exposure to the high calcium medium the tissue was quickly washed twice with a solution that contained 5×10^{-6} M EDTA and no added calcium. In a few experiments a concentration of EDTA as high as 1×10^{-4} M was added to the bathing medium. The muscle was then suspended in 10 ml of this calcium-free solution. Thereafter, the fluid was drained from the bath about every 2 min and replaced by an equal quantity of fresh calcium-free solution containing the EDTA. Almost immediately after being transferred from the high calcium to the calcium-free environment the tissue developed an appreciable increase in muscle tone. Because an external source of calcium ions was lacking, the muscle tone gradually diminished to a base line level. The time required to reach baseline varied considerably among different muscle preparations, ranging from 10 to 50 min in 15 experiments.

Two different procedures were employed to interrupt the gradual decline of muscle tone toward base line level. These were the introduction of 1.0 M ethanol or the introduction of a normal concentration of calcium chloride (1.8

mm) for a 2-min interval. Ethanol produced an inhibition of muscle tone. When the drug was removed from the bath, the spontaneous mechanical activity of the longitudinal fibers increased again, but not to the expected level. It remained partially depressed. The addition of a normal concentration of calcium ion to the medium also inhibited muscle tone, but in this case,

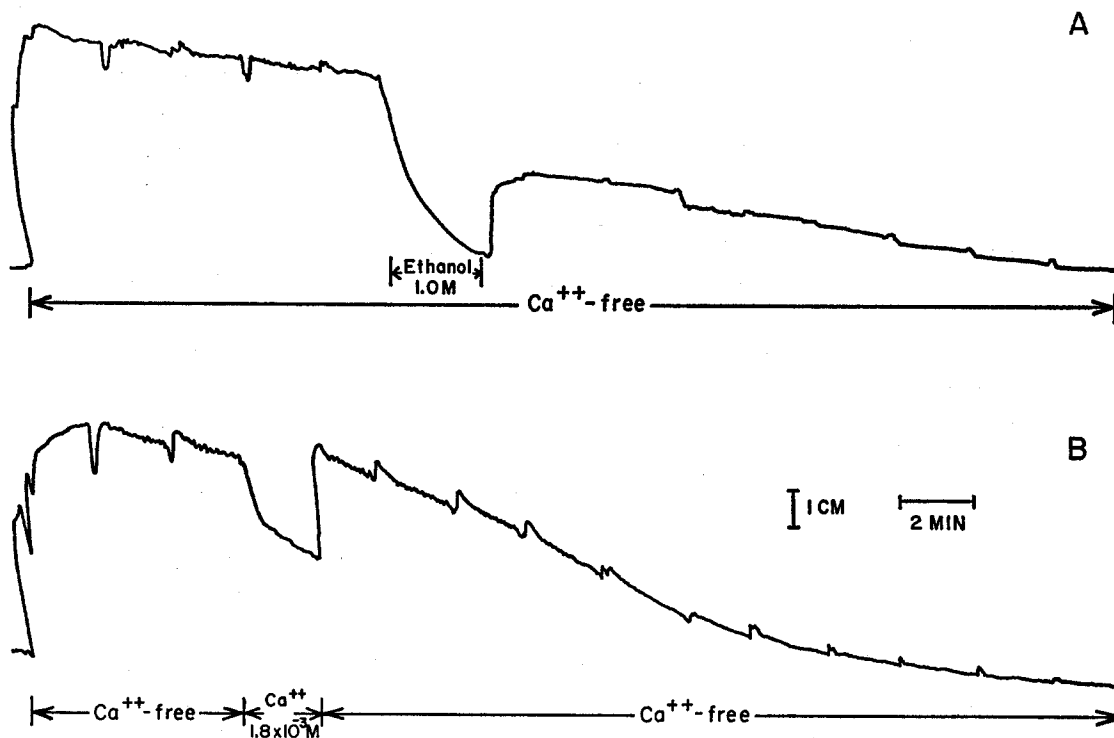


FIGURE 6 Changes in smooth muscle tone of the isolated longitudinal muscle immersed in a calcium-free medium containing 5×10^{-6} M EDTA. Prior to the introduction of the calcium-free medium, the tissues were incubated for 2 hr in a solution containing 36×10^{-8} M CaCl_2 . The small periodic changes in muscle tone seen in the records occurred during periods that the bathing fluid was replaced by fresh solution. The 2-min intervals during which ethanol or CaCl_2 was present in the bath are indicated in the diagram.

the removal of the calcium from the bath completely eliminated the inhibitory effect. These findings are represented in Fig. 6 A and 6 B.

DISCUSSION

It is generally conceded that smooth muscle like other muscle types contracts only when free calcium ions are made available to the contractile machinery (1, 5-7, 20). A smooth muscle contraction initiated by acetylcholine is thought

to take place because the neurohormone stimulates membrane reactions which enhance the inward movement of the divalent ion. Durbin and Jenkinson have postulated that a cholinergic drug enhances the inward movement of the divalent ion by increasing the permeability of the fiber membrane to calcium ions (8). Edman and Schild have contended that acetylcholine releases calcium from a bound form in the membrane (10). The evidence gathered in this study suggests that elements of both types of membrane reactions are involved.

It was assumed in interpreting our data that an agent such as calcium ion which inhibits the efflux of potassium ion from longitudinal fibers has the capacity to depress the transmembrane diffusion of other ions including calcium ion itself. The fact that this agent can inhibit the increase in smooth muscle tone induced by small concentrations of acetylcholine suggests that the permeability of the fiber membrane to calcium ion is one of the parameters modified by acetylcholine and serves to regulate the magnitude of a smooth muscle contraction.

Based on the observations made, one may visualize the following series of reactions in the muscle fiber: (*a*) calcium ions, by complexing with specific loci in the membrane, reduce the permeability of the membrane to inorganic ions; (*b*) acetylcholine counteracts this effect, perhaps by increasing membrane conductance directly, or by facilitating the development of action potentials, or both; (*c*) this permits a more rapid permeation of calcium ions through the fiber membrane; and (*d*) as a consequence muscle tone is increased.

The type of inhibition that ethanol produces in the longitudinal fibers indicates that another reaction involving calcium ion must also be considered. This drug causes a depression of an acetylcholine-induced increase in muscle tone which cannot be related to any reduction in the efflux of potassium ions. Moreover, the inhibition produced by ethanol is reversed by increasing the calcium ion concentration in the external medium; and, as an earlier study showed, it is not reversed by increasing the concentration of acetylcholine employed (18). The inference made from these data is that ethanol reduces the availability of calcium ions for contraction, but that the reaction inhibited is not the diffusion of divalent ions across the cell membrane. It is conceivable that all the interstitial calcium moving into the cell must first enter a membrane depot before it can begin its migration into the myoplasm and that ethanol modifies this initial process.

Further information was sought by experimenting with tissues that had been conditioned to develop a protracted increase in smooth muscle tone in the presence of a calcium-free medium. The pronounced increase in tone that the muscle developed in this calcium-free environment, the subsequent gradual decline of muscle tone toward base line level, and the immediate inhibition of muscle tone produced by adding 1.8 mM CaCl_2 to the medium provided

strong circumstantial evidence: (a) that the contractile machinery can be activated by calcium ions coming from a depot within the muscle fiber, presumably the fiber membrane; (b) that this store of calcium is accessible to the external medium and can be depleted by placing the muscle in a calcium-free solution; and (c) that the mobilization of calcium from the cellular depot can be inhibited by calcium ions binding a second and perhaps more superficial group of membrane loci.

The high level of muscle tone developed by the tissue in the calcium-free medium was also inhibited by ethanol. It occurred to us that the inhibition may have resulted from the capacity of the drug to deplete calcium from the membrane stores. Since in this type of experiment an external supply of calcium was eliminated, any substance which acts in this manner would be expected to abolish muscle tone irreversibly. The inhibitory effect of ethanol on muscle tone proved to be partially reversible. This suggests that only part of the total effect of ethanol may be attributed to the drug's capacity to diminish membrane stores of calcium. The availability of calcium from the internal store must also be reduced by ethanol in some other way. Conceivably, a depression of muscle tone may also occur because ethanol enters the cell and inhibits a reaction between calcium ions and the contractile elements.

Thus, the general pattern of reactions assumed to occur in longitudinal smooth muscle fibers includes the concept that calcium ions present in the extracellular space have access to two different sites or depots in the membrane. The calcium stored in one of these membrane compartments can be mobilized to activate the contractile machinery. The binding of calcium to a second and possibly more superficial site (the stabilization site) serves to decrease membrane permeability to calcium ions. As a result of a decrease in membrane permeability the diffusion of calcium from the first membrane compartment to its site of action in the myoplasm is impeded. A similar concept has been discussed previously in the literature. Daniel (5) in summarizing the work of his own group and that of a number of other investigators stated that the existence of both a superficial site (binds calcium loosely) and a sequestering site (binds calcium more tightly) in the smooth muscle membrane is favored by the evidence presently available. Furthermore, he advanced the idea that the removal of calcium from superficial binding sites could liberate calcium from sequestering sites into the myoplasm and thereby cause a contraction. Potassium and EDTA were assumed to produce a muscle contraction by depleting calcium from the superficial site. This, it was postulated, would release some calcium from sequestering sites; but, more importantly, it would promote the entry of extracellular calcium by increasing membrane permeability. Chemical agents such as adrenaline or acetylcholine were said to have an action similar to that of potassium and EDTA as well as

an additional action which causes a rapid release of calcium from both superficial and sequestering sites. In subsequent work on calcium fluxes in the rat uterus Van Breemen and Daniel found that neither a high potassium medium nor acetylcholine would increase the uptake of ^{45}Ca into the muscle fibers (24). On this basis the authors suggested that the calcium ions which activate the contractile elements must come entirely from an intracellular pool. Moreover, their experimental results were in agreement with the concept that potassium depolarization releases calcium from superficial loci, whereas a

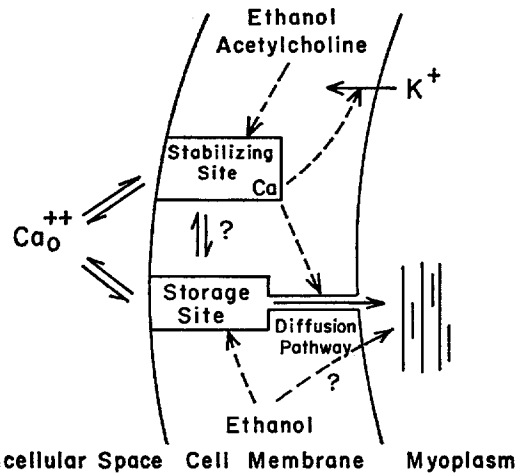


FIGURE 7. Schematic diagram of the longitudinal muscle membrane. The arrows with dashed lines point to membrane processes that are inhibited by the agents indicated. The terms Stabilizing Site and Storage Site designate the two different membrane loci that react with interstitial calcium ions. Calcium ions reacting with the stabilizing site decrease membrane permeability to inorganic ions. Calcium ions entering the storage site serve as the calcium source for the contractile machinery.

stimulatory drug releases sequestered calcium. Hinke, working with vascular smooth muscle, also drew a clear distinction between the actions of potassium and adrenaline (14). He proposed that a smooth muscle contraction initiated by a high potassium medium is activated by calcium coming from the loosely bound fraction, whereas the contraction elicited by adrenaline is activated by calcium mobilized from the tightly bound fraction.

The emphasis in this report is on the striking interrelationship between the so called superficial and sequestering sites in the longitudinal fibers from guinea pig ileum. Although acetylcholine may have complex actions on the different calcium compartments in the fiber membrane, a significant part of its stimulatory action appears to be its capacity to antagonize the membrane stabilizing action of calcium at superficial sites and to enhance, thereby, the inward migration of calcium from the less accessible membrane depot.

A schematic diagram illustrating the membrane reactions involved in translocating calcium ions in longitudinal fibers is presented in Fig. 7. The diagram includes the two proposed calcium sites of the fiber membrane which are accessible to calcium ions in the external medium. The extracellular calcium ions complexing with the stabilization site are shown to have an inhibitory effect both on the inward flow of calcium from the membrane store and on the outward flow of potassium ions. Moreover, various chemical agents which can modify the actions of calcium at each of these sites are indicated. The diagram is obviously not intended to be all-inclusive, but serves to integrate the results described in this paper and to provide a working model for future study.

This work was supported by United States Public Health Grants: 5 K3 GM-15, 209; AM 02235; 5 T1 GM 58-07; and 5 F1-GM-20, 714-03.

Received for publication 1 July 1966.

BIBLIOGRAPHY

1. BOHR, D. F. 1964. Electrolytes and smooth muscle contraction. *Pharmacol. Rev.* **16**:85.
2. BÜLBRING, E., and H. KURIYAMA. 1963. Effects of changes in ionic environment on the action of acetylcholine and adrenaline on the smooth muscle cells of guinea pig taenia coli. *J. Physiol., (London)*. **166**:59.
3. BURNSTOCK, G. 1958. The effects of acetylcholine on membrane potential, spike frequency, conduction velocity and excitability in the taenia coli of the guinea pig. *J. Physiol., (London)*. **143**:165.
4. CONWAY, E. J. 1957. Nature and significance of concentration relations of potassium and sodium ions in skeletal muscle. *Physiol. Rev.* **37**:34.
5. DANIEL, E. E. 1965. Attempted synthesis of data regarding divalent ions in muscle muscle function. In *Muscle*. W. M. Paul et al., editors. Pergamon Press Inc., New York. 295.
6. DANIEL, E. E. 1963. On roles of calcium, strontium and barium in contraction and excitability of rat uterine muscle. *Arch. Intern. Pharmacodyn.* **146**:298.
7. DANIEL, E. E. 1964. Effect of drugs on contractions of vertebrate smooth muscle. *Ann. Rev. Pharmacol.* **4**:189.
8. DURBIN, R. P., and D. H. JENKINSON. 1961. The calcium dependence of tension development in depolarized smooth muscle. *J. Physiol., (London)*. **157**:90.
9. DURBIN, R. P., and D. H. JENKINSON. 1961. The effect of carbachol on the permeability of depolarized smooth muscle to inorganic ions. *J. Physiol., (London)*. **157**:74.
10. EDMAN, K. A. P., and H. O. SCHILD. 1962. The need for calcium in the contractile responses induced by acetylcholine and potassium in the rat uterus. *J. Physiol., (London)*. **161**:424.
11. EVANS, D. H. L., H. O. SCHILD, and S. THESLEFF. 1958. Effects of drugs on depolarized plain muscle. *J. Physiol., (London)*. **143**:474.

12. FALK, G., and J. F. LANDA. 1960. Mode of action of drugs on depolarized smooth muscle. *Pharmacologist*. **2**:69.
13. FRANKENHAUSER, B., and A. L. HODGKIN. 1957. The action of calcium on the electrical properties of squid axons. *J. Physiol., (London)*. **137**:218.
14. HINKE, J. A. M. 1965. Calcium requirements for noradrenaline and high potassium ion contraction in arterial smooth muscle. In *Muscle*. W. M. Paul et al., editors. Pergamon Press Inc., New York. 269.
15. HODGKIN, A. L., and B. KATZ. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol., (London)*. **108**:37.
16. HURWITZ, L., B. TINSLEY, and F. BATTLE. 1960. Dissociation of contraction and potassium efflux in smooth muscle. *Am. J. Physiol.* **199**:107.
17. HURWITZ, L. 1960. Potassium transport in isolated guinea pig ileum. *Am. J. Physiol.* **198**:94.
18. HURWITZ, L. 1965. Calcium and its interrelations with cocaine and other drugs in contraction of intestinal muscle. In *Muscle*. W. M. Paul et al., editors. Pergamon Press Inc., New York. 239.
19. HURWITZ, L., F. BATTLE, and G. B. WEISS. 1962. Action of the calcium antagonists cocaine and ethanol on contraction and potassium efflux of smooth muscle. *J. Gen. Physiol.* **46**:315.
20. HURWITZ, L. 1961. Electrochemistry of smooth muscle and its relationship to contraction. In *Biophysics of Physiological and Pharmacological Actions*. A. M. Shanes, editor. American Association for the Advancement of Science, Washington, D. C. 563.
21. SANDOW, A. 1965. Excitation-contraction coupling in skeletal muscle. *Pharmacol. Rev.* **17**:265.
22. SHANES, A. M. 1958. Electrochemical aspects of physiological and pharmacological action in excitable cells. *Pharmacol. Rev.* **10**:59.
23. SHANES, A. M. 1958. Electrochemical aspects of physiological and pharmacological action in excitable cells. *Pharmacol. Rev.* **10**:165.
24. VAN BREEMEN, C., and E. E. DANIEL. 1966. The influence of high potassium depolarization and acetylcholine on calcium exchange in the rat uterus. *J. Gen. Physiol.* **49**:1299.
25. WEIDMAN, A. 1955. Effects of calcium ions and local anaesthetics on electrical properties of purkinje fibers. *J. Physiol., (London)*. **129**:568.
26. WEISS, G. B., R. E. COALSON, and L. HURWITZ. 1961. K transport and mechanical responses of isolated longitudinal smooth muscle from guinea pig ileum. *Am. J. Physiol.* **200**:789.
27. WEISS, G. B., and L. HURWITZ. 1963. Physiological evidence for multiple calcium sites in smooth muscle. *J. Gen. Physiol.* **47**:173.