Action of the Calcium Antagonists Cocaine and Ethanol on Contraction and Potassium Efflux of Smooth Muscle

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ABSTRACT Isolated longitudinal smooth muscle from guinea pig ileum exposed to a high potassium depolarizing medium exhibited a sustained increase in muscle tone and an increase in potassium efflux. When the concentration of calcium ion in the medium was elevated the increase in muscle tone was enhanced, but the change in potassium efflux was reduced slightly. Lowering the calcium concentration diminished the increase in muscle tone. Both cocaine and ethanol completely inhibited the sustained contraction of potassium-depolarized fibers. Addition of excess calcium ion reversed these inhibitions. Cocaine acted primarily like a competitive antagonist; and ethanol, like an indirect antagonist of calcium ion. Under certain conditions acetylcholine potentiated the reversal by calcium ion of the drug-induced inhibitions. The two inhibitory drugs had dissimilar effects on potassium efflux from smooth muscle fibers immersed in Tyrode solution. Cocaine depressed and ethanol enhanced this membrane process. However, the increase in potassium efflux induced by acetylcholine was inhibited by ethanol. This inhibition also was reversed by increasing the concentration of calcium ion in the medium. The data suggested that calcium activates and cocaine and ethanol inhibit a cellular reaction which occurs beyond the point of membrane depolarization and is essential for smooth muscle contraction. Furthermore, calcium serves to depress membrane excitability, but appears to have a specific stimulatory role in the acetylcholine-induced increase in potassium efflux from longitudinal fibers.

Calcium ion is known to play an essential role in the function of excitable tissues (1–4). In intestinal smooth muscle it must be present in order for muscle contraction to occur (5–7). Conversely, the presence of an excess of calcium ion decreases spontaneous muscle tone (8) and reduces the response of the muscle to a number of excitatory drugs (8, 9). The latter effects seem to result from a stabilization of the muscle membrane. This is evidenced by

the fact that high calcium ion concentrations have been observed to reduce spike frequency (10) and potassium efflux (11) as well as spontaneous tone. On the other hand, a reduction in external calcium ion will produce a partial depolarization of the smooth muscle membrane (12), an increase in spike frequency (10), an increase in potassium efflux (7), and a transient contraction (7, 10).

The study reported here is concerned principally with the effects of two drugs, cocaine and ethanol, which have been found to modify the actions of calcium ion on an isolated intestinal smooth muscle preparation. Experiments were carried out to elucidate the actions of the drugs both on the contractile response of the tissue and on the transmembrane efflux of potassium from the muscle cells. On the basis of the results obtained, interpretations have been made with respect to the sites of action of the drugs in the excitation-contraction pathway and the manner by which each of the drugs modifies the action of calcium ion at these sites. Previously, stimulatory and inhibitory effects of cocaine and ethanol on smooth muscle contractions have been observed (13–15). These studies, however, did not link the depressant effects of either of the drugs to a calcium antagonism. The results presented in this paper show that under specified conditions such antagonisms are readily apparent.

To some extent this study is also concerned with the physiological actions of calcium ion in smooth muscle. Experiments employing ethanol have suggested that the presence of at least trace quantities of external calcium ion are necessary for an acetylcholine-induced increase in potassium efflux to occur. By contrast, it is possible to elicit an increase in potassium efflux from smooth muscle which appears to require little or no activation by calcium ion during the response.

Some of the data reported in this paper have been presented earlier in preliminary reports (16–18).

METHODS AND PROCEDURE

The tissue used in these studies was the longitudinal smooth muscle isolated from guinea pig ileum. The manner in which the longitudinal muscle was separated from the rest of the ileum, and the histological procedure employed to estimate its purity have been described previously by Weiss *et al.* (19). The tissues used solely for studies of muscle contraction frequently contained a number of patches of circular muscle attached to the longitudinal fibers. In those experiments in which potassium efflux was also measured, care was taken to obtain longitudinal tissues that were reasonably pure (approximately 50 to 80 per cent longitudinal smooth muscle).

The bathing solution in which the muscles were suspended was a modified Tyrode solution of the following composition: NaCl 0.125 M; KCl 0.0027 M; $CaCl_2$ 0.0018 M;

$MgCl_2 \cdot 6\ H_2O$ 0.0005 M; $NaHCO_3$ 0.0238 M; glucose 0.011 M. Contractions of the smooth muscle were produced by acetylcholine or by a medium containing a high potassium ion concentration. The high potassium medium had the following composition: KCl 0.127 M; $CaCl_2$ 0.0018 M; $KHCO_3$ 0.024 M; glucose 0.011 M. A medium having this composition will be referred to as normal depolarizing solution. In some experiments a bathing medium which contained one part normal depolarizing solution and nineteen parts Tyrode solution was employed. This medium will be referred to as dilute depolarizing solution. Its composition was as follows: NaCl 0.119 M; KCl 0.0089 M; $CaCl_2$ 0.0018 M; $MgCl_2 \cdot 6\ H_2O$ 0.00048 M; $NaHCO_3$ 0.0226 M; $KHCO_3$ 0.0012 M; glucose 0.011 M. If drugs were added or the concentration of calcium ion was increased during the course of an experiment the additions were made by introducing small volumes of highly concentrated aqueous solutions of the agents used. Except for the addition of alcohol, these procedures did not change the final volume of the bathing solution by more than 2.5 per cent. The highest volume change incurred by adding alcohol during an experiment was 7.5 per cent. When high calcium ion concentrations were added to these bathing media, we often noticed a very gradual formation of insoluble salts which usually became evident several minutes after the calcium had been introduced into the solution. Moreover, the pH was affected slightly. For example, in the first 10 minutes after the $CaCl_2$ concentration was increased from 0.0018 to 0.0243 M the pH of the bathing solution decreased approximately 0.2 unit. Since, in these instances, the longitudinal muscle responded strongly and rapidly to increases in calcium ion concentration and only weakly to additions of HCl which caused comparable reductions in pH, and since conclusions drawn from the experiments did not rely on any precise estimate of the changes in calcium ion concentration, the experimental conditions employed were quite satisfactory. In one set of experiments when an accurate estimate of the calcium ion concentration was a critical factor in the experimental procedure, and it was desirable to eliminate fluctuations in pH, a special depolarizing solution was employed. To make this solution the $KHCO_3$ buffer of the normal depolarizing solution was replaced by an equimolar quantity of tris buffer (tris(hydroxymethyl) aminomethane, Sigma 121), $CaCl_2$ was added to obtain the desired final concentration, and the solution was adjusted to a pH of 7.6 with concentrated HCl.

A gas mixture consisting of 95 per cent O_2 and 5 per cent CO_2 was constantly bubbled through the bathing solutions which contained bicarbonate ion. Pure oxygen was bubbled through the solution containing tris buffer. The temperature of the bath was kept at 31.5–32.5°C.

The tissues suspended in the muscle bath were attached to a lever which recorded isotonic contractions of the longitudinal fibers on a kymograph. Changes in muscle length were magnified by the lever 3.6 times. Tension on the muscle was approximately 0.35 gm. Contraction studies were performed in a 40 ml bath.

The radioactive tracer K^{42} was used to measure potassium efflux. The rate of escape of K^{42} from a radioactive muscle preparation was estimated by measuring the increase in number of counts per unit time that appeared in the bathing fluid. Details of the experimental procedure have been described previously (7).

RESULTS

The results illustrated in the figures of this paper, in each case, were taken from single representative experiments. All measurements of smooth muscle contractions were repeated a minimum of eight times on tissues that were obtained from different animals. Although the muscle preparations tested were found to vary somewhat in their sensitivity and speed of reaction to the drugs

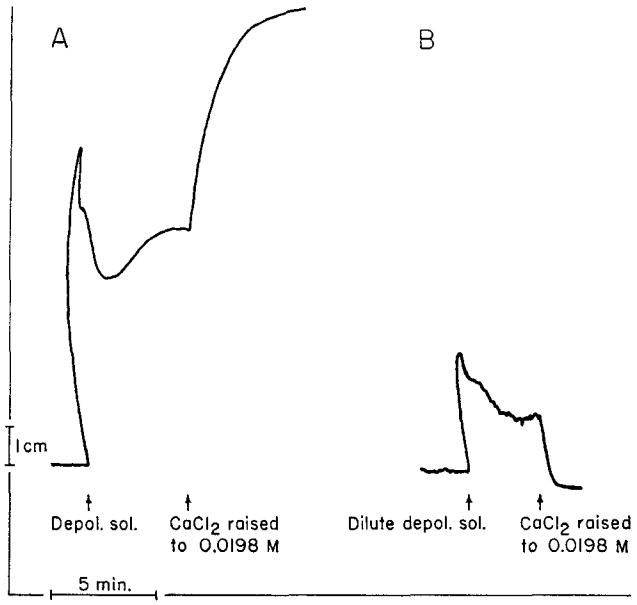


FIGURE 1. Isotonic contractions of isolated longitudinal smooth muscle. Points at which designated substances were introduced into the muscle bath are indicated by arrows. Depol. Sol. refers to normal depolarizing solution, and Dilute Depol. Sol. refers to dilute depolarizing solution.

and ions introduced, qualitative changes in muscle tone produced by the several agents used were clearly evident and highly reproducible. Because of the variability often present among the contractile responses of a single muscle preparation some difficulty was encountered in making an accurate quantitative comparison of the effect of different concentrations of inhibitory drugs and calcium ion on the level of smooth muscle tone of a tissue. Data used for such comparisons were taken from experiments in which a series of responses of a tissue elicited by the drugs and ions used exhibited minimal random variation.

Each study of potassium efflux that is presented was carried out on at least five different tissues. The quantitative results obtained in these experiments are given in the text and table below.

Influence of Ca Ion on Contraction of K-Depolarized Muscle To produce increases in smooth muscle tension of the taenia coli of the guinea pig and the uterus of the rat with a depolarizing solution that contains a high potassium concentration, calcium ion must also be present in the medium (20, 21). The same holds true for the longitudinal smooth muscle of guinea pig ileum. This is partly indicated in Fig. 1 A where it is seen that the maintained contraction of the ileal muscle produced by normal depolarizing solution is strengthened by raising the external concentration of calcium ion. Contractions of the muscle are also weakened by lowering the calcium ion concentra-

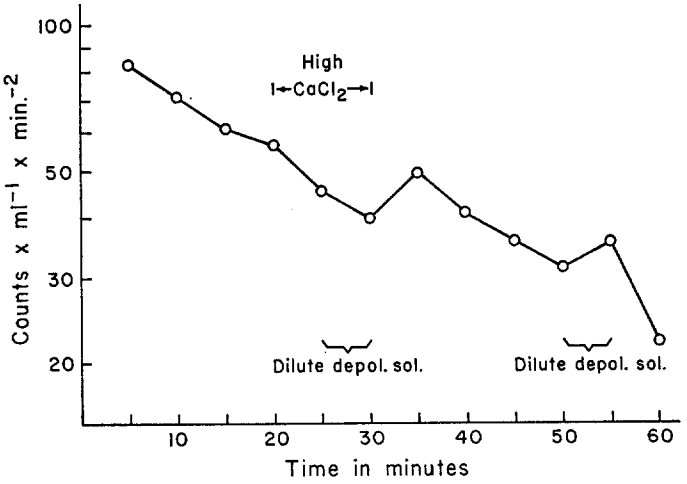


FIGURE 2. Potassium efflux from isolated longitudinal smooth muscle. A 0.0198 M concentration of $CaCl_2$ (high $CaCl_2$) was present in the bathing solution for the 10 minute period indicated by the arrows. At all other times the normal concentration of calcium chloride (0.0018 M) was present. Dilute depolarizing solution (Dilute Depol. Sol.) was introduced for the 5 minute periods indicated by the braces.

tion. The action of this divalent ion, however, is not exclusively a stimulatory one. Fig. 1 B shows that dilute depolarizing solution still causes a shortening of the muscle, but to a lesser degree. In this instance increasing the calcium ion concentration from 0.0018 to 0.0198 M, which had the effect of augmenting the muscle response obtained in normal depolarizing solution, strongly inhibited the response evoked by the dilute depolarizing solution. Calcium ion thus exhibits both stimulatory and depressant actions on the potassium-induced contractions of the isolated longitudinal smooth muscle.

Influence of a High Ca Ion Concentration on Potassium Efflux of K-Depolarized Muscle We have studied the changes in potassium efflux which the dilute and normal depolarizing solutions elicit in the presence of a normal and an elevated concentration of calcium ion. In eight experiments in which a tissue

was immersed in dilute depolarizing solution (normal Ca ion) for a 5 minute period a 22 to 56 per cent increase in the rate of outward migration of potassium ion as well as a small increase in muscle tone was observed. Both effects were strongly inhibited by increasing the concentration of calcium ion to 0.0198 M (Figs. 2 and 1 B). In normal depolarizing solution (normal Ca ion) a 311 to 647 per cent increase in the efflux of potassium ion and a large increase in muscle tone were observed (Table I and Fig. 1 A). When the calcium ion concentration was raised to 0.0198 M, muscle tone was further increased but a comparable change in the efflux of the potassium ion was not observed. In fact, the sharp increase in potassium efflux which occurred in the normal

TABLE I

	Percentage increase in K^{42} efflux in paired tissues exposed to depolarizing solution for a 5-minute period. Both members of a pair were obtained from the same animal		
Experiment No.	Member of pair exposed to depolarizing solution containing 0.0018 M Ca^{++}	Member of pair exposed to depolarizing solution containing 0.0198 M Ca^{++}	Percentage change in the increase of potassium efflux resulting from the elevation of Ca^{++} concentration
1	647	333	−48.5
2	471	371	−21.2
3	563	378	−32.9
4	597	452	−24.3
5	311	278	−10.6
6	469	556	+18.6
7	572	412	−28.0
8	647	587	−9.3
9	542	488	−10.0

The increase in $CaCl_2$ concentration reduced the increase in K^{42} efflux by a mean of 18 per cent with an SE of 6 per cent. $0.01 < P < 0.05$.

The average rate constant for the efflux of K^{42} in Tyrode solution was 0.033 $min.^{-1}$ with a standard deviation of 0.011 $min.^{-1}$. Differences in rate constants between members of a pair were found to be 26 per cent or less.

depolarizing solution was slightly inhibited. The results of nine experiments on paired tissues are given in Table I. Thus, under the several conditions employed, an elevated concentration of calcium ion was shown to be a consistent inhibitor of transmembrane efflux of potassium, but displayed either stimulatory or inhibitory actions toward muscle tone.

Effects of Cocaine and Ethanol on Contraction of K-Depolarized Muscle The high level of sustained muscle tone which the longitudinal muscle attains in a depolarizing solution is completely depressed by the addition of appropriate concentrations of cocaine or ethanol. Figs. 3 A and 4 A show the profound depression of muscle tone which 4.4×10^{-4} M cocaine produces in one depolarized tissue, and 0.54 M ethanol in another. A characteristic difference

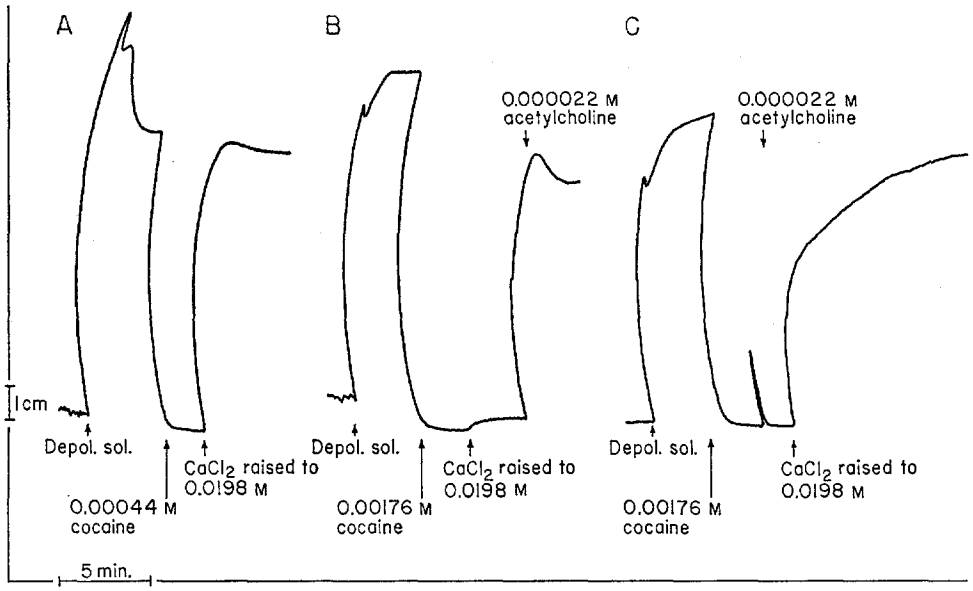


FIGURE 3. Isotonic contractions of isolated longitudinal smooth muscle. Points at which designated substances were introduced into the muscle bath are indicated by arrows. Depol. Sol. refers to normal depolarizing solution.

between the actions of the two drugs is the initial increase in muscle tone which ethanol elicits prior to the development of the inhibitory effect. This transitory stimulation is lacking in the action of cocaine on depolarized smooth muscle. The same figures indicate that reversal of the inhibitory effects produced by the two drugs may be accomplished simply by increasing the concentration of calcium ion in the bathing solution. Depression of muscle tone

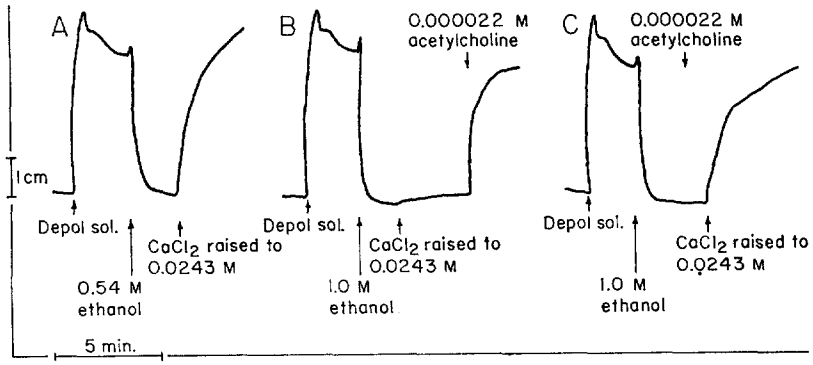


FIGURE 4. Isotonic contractions of isolated longitudinal smooth muscle. Points at which designated substances were introduced into the muscle bath are indicated by arrows. Depol. Sol. refers to normal depolarizing solution.

can also be reversed by replacing the solution containing the inhibitory drugs with fresh bathing solution.

Calcium-Drug Antagonism Since these experiments suggested an antagonism between calcium ion and the two inhibitory drugs on smooth muscle tone, further attempts were made to substantiate and, in some measure, to characterize this phenomenon. It is apparent from the tracings in Fig. 5 A, B, C, and D that calcium ion does indeed antagonize the actions of cocaine and ethanol. The degree of relaxation of the smooth muscle brought about by either 1.1×10^{-4} M cocaine or 0.43 M ethanol was significantly greater in

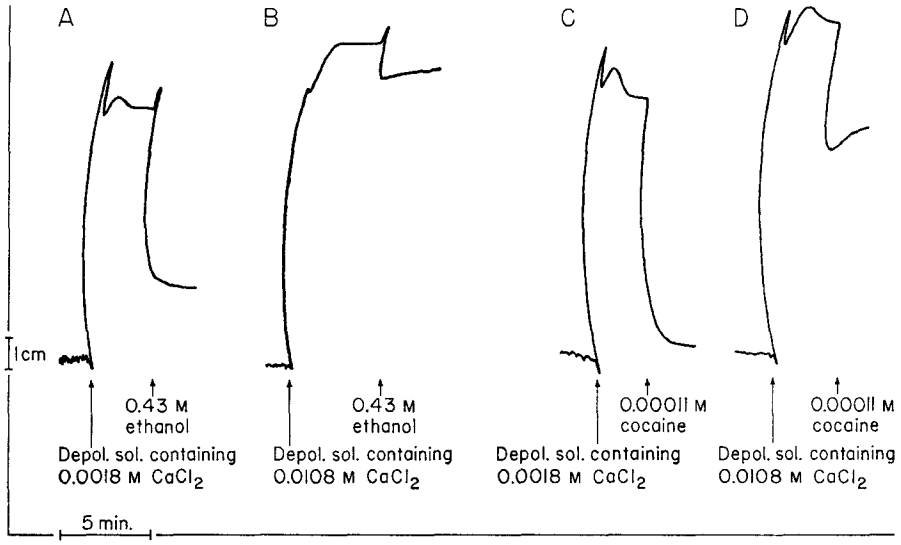


FIGURE 5. Isotonic contractions of isolated longitudinal smooth muscle. Points at which designated substances were introduced into the muscle bath are indicated by arrows. Depol. Sol. refers to depolarizing solutions. These solutions contained the concentrations of $CaCl_2$ indicated.

normal depolarizing solution than in a depolarizing solution that contained six times the normal concentration of calcium ion.

To obtain some insight into the type of antagonism that exists between calcium ion and each of the inhibitory agents, contraction experiments were performed in which an ileal preparation was immersed in depolarizing solution, and arbitrarily chosen concentrations of calcium ion and an inhibitory agent were added. When the muscle responded the depolarizing solution was washed out and, after a period of rest in Tyrode solution, the tissue was resuspended in depolarizing solution and quite different concentrations of calcium ion and inhibitory agent were introduced. Care was taken to modify the concentrations of calcium ion and its antagonist by the same factor so that

the ratio of their concentrations remained constant. When cocaine was used as the antagonist, a threefold increase in the concentrations of calcium ion and cocaine did not alter appreciably the final level of muscle tone which the longitudinal fibers assumed. On the other hand, when ethanol was the antago-

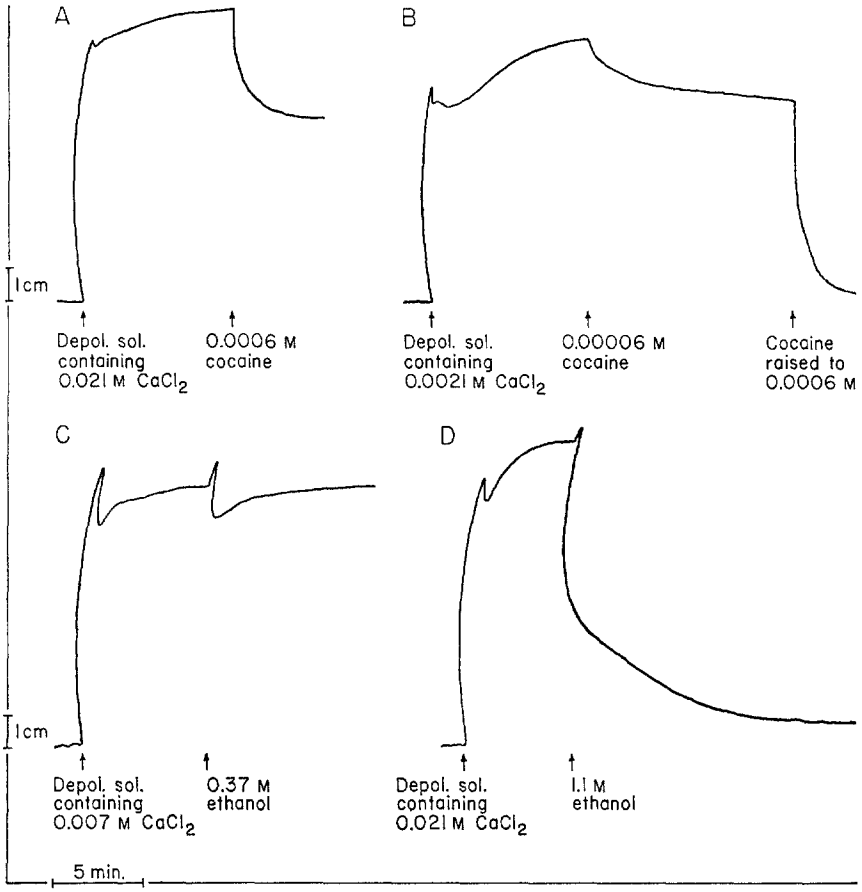


FIGURE 6. Isotonic contractions of isolated longitudinal smooth muscle. Points at which designated substances were introduced into the muscle bath are indicated by arrows. Depol. Sol. refers to depolarizing solutions that were buffered with tris ion. These solutions contained the concentrations of $CaCl_2$ indicated.

nist a threefold increase in the concentrations of both calcium ion and ethanol resulted in a much lower level of muscle tone. The ways in which the longitudinal muscle responded to the changes in the concentrations of calcium ion and cocaine, and to similar changes in the concentrations of calcium ion and ethanol, support the concepts that cocaine acts as a competitive antagonist of calcium ion, and ethanol as an indirect antagonist of calcium ion. Theo-

retical considerations require that an experimental procedure of the type just described be performed using relatively high concentrations of the agonist and antagonist so that almost all the "tissue receptors" involved are saturated with one or the other agent. This being the case, it is an obvious disadvantage to work with a bicarbonate buffer which can react with calcium ion that is present in high concentrations and thereby diminish the effective concentration of the divalent ion. Consequently, the experimental results obtained with the bicarbonate-buffered solutions were verified by performing similar experiments in depolarizing solutions buffered with tris ion.

In eight experiments performed with the tris-buffered depolarizing solutions the final level of muscle tone which the longitudinal tissue assumed in the lower concentrations of calcium (0.0021 M) and cocaine (0.00006 M) was somewhat above that attained by the tissue in the presence of tenfold higher concentrations of these agents. In three experiments a tenfold change in calcium and cocaine concentrations caused no change in final level of muscle tone. These findings may indicate that a small component of the inhibition produced by cocaine is not competitive with respect to calcium ion. However, the major inhibitory action of cocaine seems to be reversed by calcium ion in a competitive manner. This is illustrated in Fig. 6 A and B. One may observe that the level of muscle tone determined by the presence of 0.0021 M calcium ion and 0.00006 M cocaine (Fig. 6 B) is not very different from the level of muscle tone determined by tenfold higher concentrations of both these agents (Fig. 6 A), whereas the depression of muscle tone produced by the high concentration of cocaine is much greater in the presence of the low calcium ion concentration (Fig. 6 B) than it is in the presence of the high calcium ion concentration (Fig. 6 A).

As in previous experiments performed with the bicarbonate-buffered solutions, a threefold increase in the concentrations of calcium ion and ethanol produced a much lower final level of muscle tone (Fig. 6 C and D).

In preliminary publications (16, 18) the statement was also made that a tenfold increase in both calcium and cocaine concentrations did not alter appreciably the final level of muscle tone of the longitudinal muscle. These experiments were carried out with concentrations of calcium ion ranging from 0.007 to 0.07 M. Subsequent work showed that the introduction of sucrose in a concentration that approached the osmolarity of the highest $CaCl_2$ concentration used in the bathing media interfered noticeably with the inhibition of muscle tone by cocaine. Consequently some difficulty is encountered in interpreting the nature of the calcium-cocaine antagonism from the results of these experiments. This difficulty has been overcome in the experiments reported on in this paper. Sucrose, in quantities (up to 0.034 M) calculated to approximate or exceed the osmolarity of 0.0189 M $CaCl_2$ in depolarizing solution, was added to bathing media containing 0.0021 M $CaCl_2$. It was found

that these concentrations of sucrose produced either no change or slight increases in the final levels of muscle tone of tissues that were partially depressed by cocaine and small decreases in the final tone of smooth muscles that were partially depressed by ethanol.

Acetylcholine Potentiation The antagonisms between calcium ion and the two inhibitory drugs are also influenced by acetylcholine. It may be seen in Figs. 3 A and B, and 4 A and B, that the respective concentrations of calcium ion which overcome the depression of muscle tone produced by 4.4×10^{-4} M cocaine and 0.54 M ethanol do not elicit more than a slight reversal of the

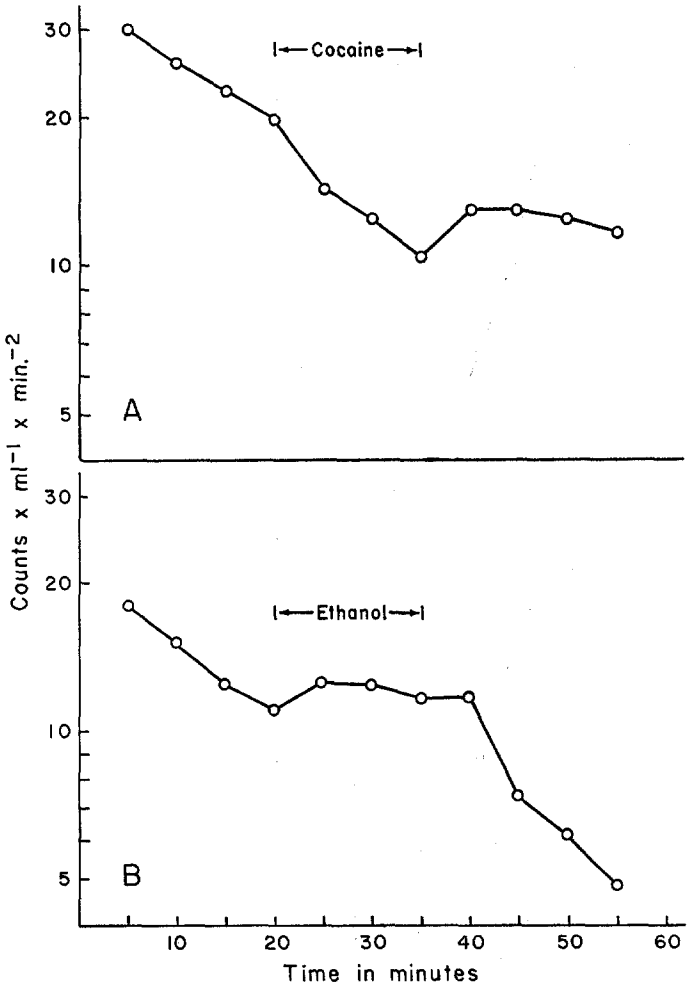
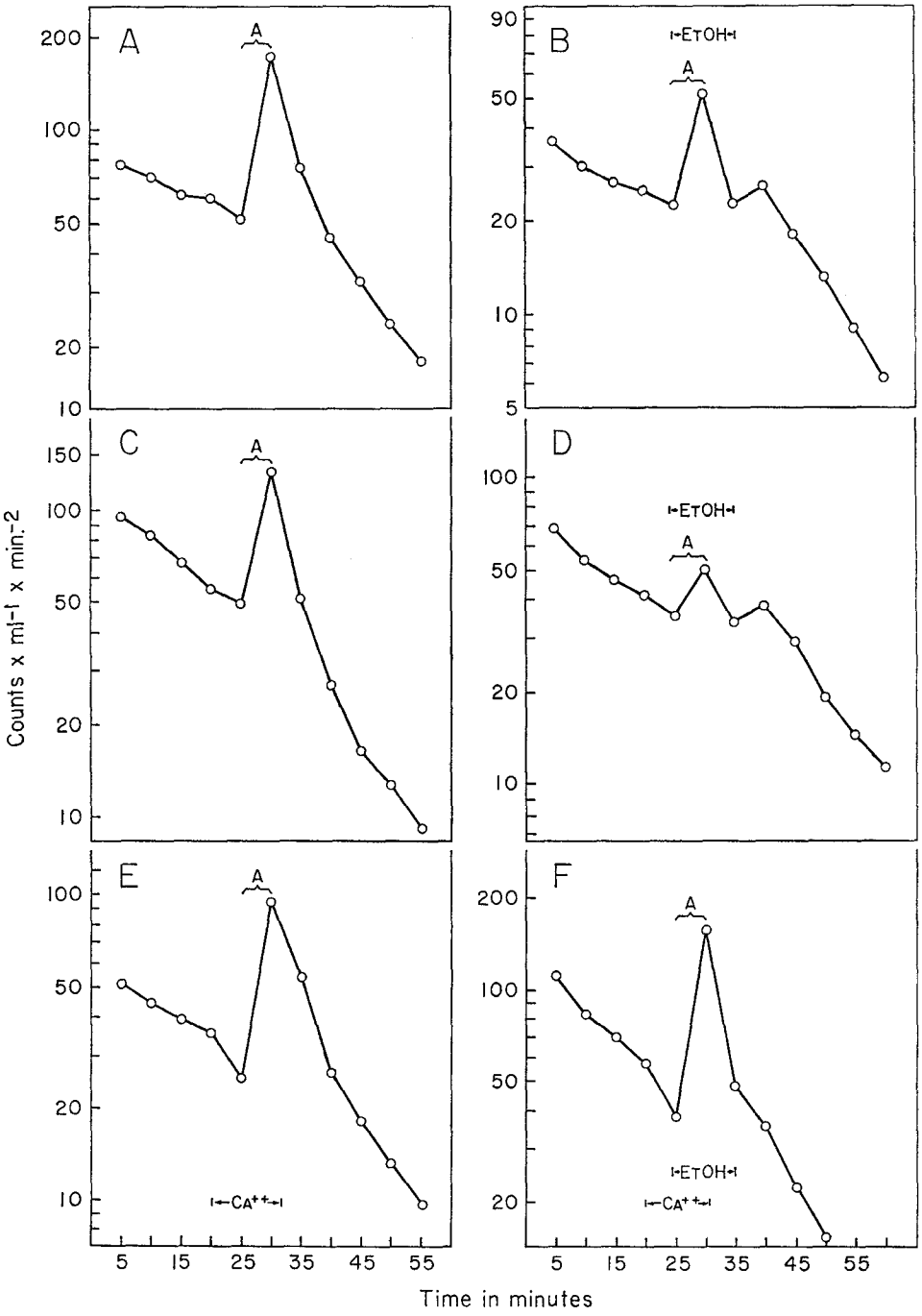


FIGURE 7. Potassium efflux from isolated longitudinal smooth muscle. Cocaine (3.9×10^{-3} M) or ethanol (1.2 M) was added to the bathing solution for the 15 minute interval indicated by the appropriate arrows.


A
B
C
D
E
F
A
ETOH
CA++
Counts x ml-1 x min.-2
Time in minutes
5
15
25
35
45
55

depression produced by 17.6×10^{-4} M cocaine and 1.0 M ethanol. However, appreciable restoration of muscle tone may still be achieved by the subsequent addition of 2.2×10^{-5} M acetylcholine. That is to say, calcium ion, within the concentration ranges employed in these experiments, reversed the partial or complete inhibition of muscle tone caused by small to moderate concentrations of cocaine or ethanol but did not effectively overcome the inhibition produced by large amounts of the inhibitory agents unless it was potentiated by acetylcholine. The addition of acetylcholine in the absence of an elevated calcium ion concentration (Figs. 3 C and 4 C) was also ineffective in reestablishing a sustained high level of tone, although small transient contractions often occurred. Even partial depression of muscle tone by cocaine or ethanol in the depolarized ileal muscle is not reversed by the addition of acetylcholine alone.

Effects of Cocaine and Ethanol on Potassigm Efflux of Unexcited Muscle Although cocaine and ethanol are both inhibitors of smooth muscle tone, their actions on the transmembrane efflux of potassium ion from an unexcited longitudinal muscle immersed in normal bathing fluid are dissimilar. In five experiments 3.9×10^{-3} M cocaine was observed to depress potassium efflux from 10 to 37 per cent, whereas 1.2 M ethanol elevated potassium efflux 22 to 35 per cent. Fig. 7 A and B illustrates these effects. We have found that ethanol also enhances potassium efflux in a bathing solution that contains only one-fifth normal calcium ion concentration.

Effects of Ethanol on Acetylcholine-Induced Potassium Efflux It is interesting that the same concentration of ethanol that elevates the potassium efflux of an unexcited longitudinal smooth muscle has an opposite effect on the increase in potassium efflux brought about by acetylcholine. In Fig. 8 A and B, one may observe the changes in potassium efflux initiated by acetylcholine both in the absence and presence of ethanol. The results of five experiments showed that the average inhibition of this ion transport process by 1.2 M ethanol was 46 ± 11 per cent. When the entire experimental procedure was repeated in a bathing solution containing one-fifth normal calcium ion concentration, the effect of ethanol was magnified (Fig. 8 C and D). The inhibition averaged 71 ± 5 per cent. On the other hand, a high calcium ion concentration (six

FIGURE 8 *opposite.* Potassium efflux from isolated longitudinal smooth muscle. A 1.1×10^{-5} M concentration of acetylcholine (A) was added to the bathing solution for the 5 minute intervals indicated by the braces A. 1.2 M concentration of ethanol (ETOH) was added to the bathing solution for the 10 minute intervals indicated by the appropriate arrows. In A and B the bathing solutions contained normal calcium ion concentration (0.0018 M). In C and D the bathing solutions contained one-fifth normal calcium ion concentration (0.00036 M). In E and F the bathing solutions contained six times normal calcium ion concentration (0.0108 M) for the 10 minute intervals designated by the arrows.

times normal) completely reversed the inhibition by ethanol (Fig. 8 E and F). Smooth muscle contractions elicited by acetylcholine are similarly inhibited by ethanol and reactivated by high concentrations of calcium ion (11).

DISCUSSION

Our investigation has shown that the effects of cocaine and ethanol on intestinal smooth muscle function can be ascribed either to an intensification of or to an interference with the physiological actions of calcium ion. This relationship first became apparent from studies of the effects of calcium, cocaine, and ethanol on the sustained contraction of the ileal muscle produced by a high potassium medium. The data indicated that the height of contraction of the muscle was dependent on the calcium ion concentration in the medium and that the action of calcium ion on muscle tone was antagonized by cocaine and ethanol. Cocaine behaved primarily as a competitive antagonist of calcium ion. Ethanol appeared to antagonize calcium ion in an indirect manner.

An effort was made to localize somewhat the site of interaction of the drugs and the divalent ion. By analogy with many experimental findings obtained on other excitable tissues, we assumed that the medium containing a high potassium ion concentration acts to depolarize partially the longitudinal muscle membrane. The depolarized membrane in turn initiates one or more reactions which eventually activate the contractile mechanism. In this study a modification of the increase in potassium efflux induced by normal depolarizing solution is considered to be an indication of an effect on the level of depolarization of the muscle membrane. Modifications of muscle tone are considered to be a measure of the functional changes which occur in the subsequent steps involved in contraction. Based on these assumptions the increase in muscle tone produced by an elevation of the calcium concentration results from an enhancement of reactions which occur beyond the initial stage of depolarization. This was borne out by the fact that the addition of excess calcium ion depressed slightly the efflux of potassium ion while it increased muscle tone. When tested in dilute depolarizing solution excess calcium ion appeared to have a more profound depressant effect on the smooth muscle membrane. The result was an inhibition, not only of the increase in potassium efflux, but also of the increase in muscle tone which normally follows.

Because the depression of muscle tone of potassium-depolarized fibers by cocaine and ethanol can be reversed by high concentrations of calcium ion, these two drugs also are believed to exert their effects on some reaction in the excitation-contraction pathway which takes place subsequent to membrane depolarization. With respect to cocaine this conclusion is further supported by the competitive nature of the antagonism between this drug and

calcium ion. However, in a previous publication it was shown that cocaine reduces the increase in potassium efflux induced by depolarizing solution (19). Thus one cannot eliminate the possibility that inhibition of contraction by cocaine may be due, at least in part, to a reduction in membrane depolarization. With ethanol this latter possibility is less likely since Weiss and Hurwitz (11) have found that the increase in potassium efflux induced by normal depolarizing solution is not diminished by ethanol. The evidence favors the postulate that depression of muscle tone of a potassium-depolarized tissue by ethanol results entirely from the action of the drug on a calcium-dependent step beyond the point of membrane depolarization. Some investigators have postulated that an inward release of ionized calcium from membrane sites constitutes the process which couples excitation to contraction (3, 22, 23). Possibly cocaine and ethanol affect this event.

Calcium ion has also been observed to modify the potassium efflux of the unexcited longitudinal smooth muscle fiber. A large increase in the concentration of calcium ion lowers (11) while a reduction in concentration elevates (7) the rate of outward movement of potassium ion. The depressant effect of cocaine and the stimulatory effect of ethanol on this ion transport process of the unexcited tissue may also be associated with an action of calcium ion. In fact, the manner by which these drugs alter the potassium efflux of the unexcited tissue and the manner by which they inhibit muscle tone of the potassium-depolarized tissue provide a reasonable explanation for the modes of action of the drugs. Cocaine, by competitively replacing calcium ion at specific tissue and/or membrane sites, could deplete calcium ions needed for contraction and at the same time simulate the action of a high level of calcium ion on ion movements. Ethanol, by indirectly blocking the action of calcium ion or displacing the divalent ion from tissue sites, could effectively reduce the quantity of tissue calcium that can be utilized to activate muscle contraction, and by the same token, reduce the calcium ion required to restrict the outward movement of potassium ion.

Previously Burridge had obtained some evidence of a calcium-cocaine antagonism in heart muscle (24). He found that cardiac muscle became more susceptible both to the stimulatory and inhibitory actions of cocaine on contraction when the concentration of calcium ion in the medium was decreased. Shanes *et al.*, studying the effects of calcium and cocaine on the electrical characteristics of the squid axon, found that both agents raised the threshold of membrane excitability, whereas calcium augmented and cocaine depressed increases in sodium and potassium conductances during activity (25). The authors state that their data support the proposal that cocaine and calcium ion act at different loci. In this study the results obtained on smooth muscle are more consistent with the postulate that cocaine and calcium compete for the same loci in the cell. An interrelationship between cocaine and

calcium ion has also been uncovered by purely chemical procedures. Berwick has found that cocaine causes a loss of calcium ion from skeletal muscle fibers (26).

In work performed with ethanol, Gallego noted that the drug would depolarize frog sciatic nerve (27). In addition, Knutsson found that it decreased membrane resistance and lowered membrane potential in frog sartorius muscle (28). Presumably the increase in potassium efflux which ethanol evokes in our preparation represents an analogous effect on smooth muscle. It would be interesting to examine whether or not a blockade or displacement of calcium ion was a common denominator in all these effects of ethanol.

An additional aspect of the antagonism between calcium ion and the two inhibitory drugs, investigated only briefly, is the potentiation of the action of calcium by acetylcholine. This potentiation was observed only in the presence of elevated concentrations of calcium ion and under conditions in which the level of the divalent ion was still not high enough to produce an appreciable reversal of depressed muscle tone. It may be that cocaine and ethanol inhibit one tissue reaction which can be activated by calcium ion alone and another which requires the presence of both calcium and acetylcholine to become activated.

The experiments discussed above were performed to determine where and in what manner calcium and its antagonists interact. The discussion which follows is concerned with a physiological action of calcium ion in the longitudinal muscle. By virtue of its proposed antagonism toward calcium ion, ethanol was useful for revealing what appears to be a distinct difference in dependence on calcium ion between the transmembrane potassium movement of an unexcited tissue and that of an acetylcholine-treated tissue.

Previous work has shown that the contraction which pilocarpine elicits in the longitudinal muscle is far more sensitive to calcium deprivation than is the accompanying increase in potassium efflux which the drug evokes (7, 19). The same observations have been made for acetylcholine-induced responses of the smooth muscle (11). In a medium which is almost free of calcium ion the muscle will eventually lose its power to contract while, during that same period of time, the increase in potassium efflux which acetylcholine initiates remains essentially unaffected. However, the inhibition of the acetylcholine-induced increase in potassium efflux by 1.2 M ethanol, and the reversal of this effect by high levels of calcium ion suggest that at least a small quantity of calcium ion must be present in the medium in order to obtain an increase in potassium efflux with acetylcholine.

In sharp contrast to its inhibitory effect on the drug-induced ion transport process, ethanol enhances the rate of outward movement of potassium ion in the unexcited tissue. It was suggested in earlier publications that the changes in potassium movements elicited by pilocarpine and acetylcholine

were possibly the reflection of a change in permeability to another ion which depolarizes the membrane (19, 29). Results in this study support the suggestion that acetylcholine initiates a calcium-dependent reaction which is associated with the observed increase in potassium efflux elicited by the drug and which probably has little if any relationship to potassium movements in the unexcited smooth muscle. It is not possible at present to define this calcium-dependent reaction. It may be a permeability change to an inorganic ion, the binding of acetylcholine to a tissue receptor, or perhaps some other membrane reaction. The experimental data indicate, however, that calcium ion, in addition to its stimulatory effect on a process essential for contraction and its depressant effect on membrane excitability, appears to play a specific stimulatory role in the acetylcholine-induced increase in potassium efflux from longitudinal smooth muscle fibers.

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REFERENCES

1. BRINK, F., *Pharmacol. Rev.*, 1954, **6**, 243.
2. SHANES, A. M., *Pharmacol. Rev.*, 1958, **10**, 59.
3. SHANES, A. M., *Pharmacol. Rev.*, 1958, **10**, 165.
4. FRANKENHAEUSER, B., and HODGKIN, A. L., *J. Physiol.*, 1957, **137**, 218.
5. MAGEE, H. E., and REID, C., *J. Physiol.*, 1927, **63**, 97.
6. CRAVER, B. N., and BARRETT, W. E., *Am. J. Digest. Dis.*, 1951, **18**, 163.
7. HURWITZ, L., TINSLEY, B., and BATTLE, F., *Am. J. Physiol.*, 1960, **199**, 107.
8. AMBACHE, N., *J. Physiol.*, 1946, **104**, 266.
9. GRAHAM, J. D. P., *J. Physiol.*, 1952, **116**, 36P.
10. HOLMAN, M. E., *J. Physiol.*, 1958, **141**, 464.
11. WEISS, G., and HURWITZ, L., unpublished data.
12. BURNSTOCK, G., and STRAUB, R. W., *J. Physiol.*, 1958, **140**, 156.
13. FELDBERG, W., and LIN, R. C. Y., *Brit. J. Pharmacol.*, 1949, **4**, 33.
14. CARLSTRÖM, B., *Skand. Arch. Physiol.*, 1926, **48**, 8.
15. GUILLOT, M., and GWAN, O. S., *Compt. rend. Soc. biol.*, 1937, **125**, 33.
16. HURWITZ, L., BATTLE, F., and WEISS, G. B., *Pharmacologist*, 1961, **3**, 67.
17. HURWITZ, L., *in* Biophysics of Physiological and Pharmacological Actions, (A. M. Shanes, editor), Washington, D. C., American Association for the Advancement of Science, 1961, 563.
18. HURWITZ, L., BATTLE, F., and WEISS, G. B., *J. Gen. Physiol.*, 1962, **45**, 604A.
19. WEISS, G. B., COALSON, R. E., and HURWITZ, L., *Am. J. Physiol.*, 1961, **200**, 789.
20. DURBIN, R. P., and JENKINSON, D. H., *J. Physiol.*, 1961, **157**, 90.
21. EDMAN, K. A. P., and SCHILD, H. O., *J. Physiol.*, 1961, **155**, 10P.
22. SANDOW, A., *Yale J. Biol. and Med.*, 1952, **25**, 176.

23. BIANCHI, C. P., and SHANES, A. M., *J. Gen. Physiol.*, 1959, **42**, 803.
24. BURRIDGE, W., *J. Pharmacodyn.*, 1922, **26**, 115.
25. SHANES, A. M., FREYGANG, W. H., GRUNDFEST, H., and AMATNIEK, E., *J. Gen. Physiol.*, 1959, **42**, 793.
26. BERWICK, M. C., *J. Cell. and Comp. Physiol.*, 1951, **38**, 95.
27. GALLEGO, A., *J. Cell. and Comp. Physiol.*, 1948, **31**, 97.
28. KNUTSSON, E., *Acta Physiol. Scand.*, 1961, **52**, 242.
29. HURWITZ, L., *Am. J. Physiol.*, 1960, **198**, 94.