EFFECT OF DRUGS ON CONTRACTIONS OF VERTEBRATE SMOOTH MUSCLE[1]

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Introduction

Activation of smooth muscle to contract or to relax is usually considered as a property of the interactions between chemical mediators or their analogs and the cell surface (see Furchgott, p. 21 this volume). Specialized and different sites on the cell surface appeared to be involved in the response to each mediator. The selectivity of each of these types of site for certain types of chemical structures and the competitive kinetics between structurally related substances which was often found suggested that ability to interact with each type of site was determined by its chemical structure. Sites reacting with chemicals of a class were designated as a corresponding class of receptors. The kinetics of receptor-drug interactions has become a highly specialized area and many additional and controversal complexities have been elaborated (1, 2, 3).

Advances in the electrochemistry of nerve and of skeletal muscle gradually have been extended to smooth muscle, and certain interesting differences have appeared. Similar techniques revealed smooth muscles to have slightly lower resting potentials (4 to 14), to be sometimes depolarized by stretch (4, 5, 14, 15), to have transmembrane action potentials relatively insensitive to removal of external sodium (4 to 7, 14, 16, 17, 18), and during rest to be relatively insensitive to increases in external potassium (4 to 6, 8, 10, 19) etc. However, the two results that must ultimately lead to a revolution in our thinking about the electrochemistry of smooth muscle have been the findings that the fluxes of $^{22}Na^+$ or $^{24}Na^+$ are many times faster than those of $^{42}K^+$ (20 to 24),[2] and that smooth muscles can respond to chemical stimulants by contraction or relaxation though completely depolarized (21 to 39, 22). It is the latter result and its consequences that this review will examine in considerable detail, though, consideration will also be given the interrelations of these with other findings with regard to smooth muscle.

The point of view will be taken and evidence (40) reviewed that both depolarized and polarized smooth muscle is activated by release of Ca^{++} from the cell surface increasing local activity and permitting Ca^{++} entry into the cell interior. The cell surface probably has the properties of an ion exchanger.

[1] This review covers literature available up to July 15, 1963.

[2] A number of investigators (25, 26) appear to have missed the early rapid sodium fluxes owing to the practices of discarding of effluent for 15 to 60 minutes during efflux and of soaking tissues in nonradioactive solutions for similar periods as a preliminary to counting the tracer uptake.

Some substances act directly to influence this calcium exchange (ATP, EDTA, Mg) while others appear to act indirectly by influencing the affinity of the negative binding sites for calcium and other divalent cations (potassium, acetylcholine, adrenaline, histamine, etc.); and a final group of drugs (ouabain, NaF, cocaine) appear to act by their interference with membrane transport processes, preventing Ca^{++} binding and transport. Finally, the suggestion will be made that the properties of this ion exchange site suggest it must be closely related to the membrane Mg^{++} and (Na^{+} and K^{+}) activated ATPase.

This reviewer does not pretend to offer comprehensive coverage of all aspects of drug actions on smooth muscle in the limited space available. Some other reviews in this field have appeared recently covering autonomic nerve transmission to smooth muscle (14), and a recent monograph from a symposium has covered many aspects of the field (41). Agonist-antagonist interrelations are covered elsewhere, in this volume (p. 21). No mention will be made of the recent work on local effects of altered ion concentration on vascular muscle or on the numerous polypeptides (other than oxytocin) which affect smooth muscle. Only vertebrate smooth muscle will be considered.

The process of activation of smooth muscle—an overall view.—The drugs of interest to this review are those which initiate, accelerate, or otherwise aid the process leading to contraction, and those which prevent or antagonize this process. Those antagonists which act selectively at receptors will be mentioned only in passing. Although a variety of receptors are available for the initiation of contraction it would be contrary to what we expect of the efficiency of biological engineering to find that a combination of a drug at each receptor initiates a unique, independent chain of events leading to contraction or relaxation. It is likely that most of such chains are common to all. We are not surprised then to find that depletion[3] of smooth muscle calcium reversibly prevents all contractile responses (14, 17, 19, 22, 25, 30, 31, 33, 34, 36–40, 42–57) and that certain substances (e.g., papaverine) can prevent or diminish contractile responses to all or nearly all stimulants (58, 59). Further, we are not surprised that certain substances (e.g. Ba^{++}, K^{+}) cause contractures of all or nearly all smooth muscles (58, 60 and many others) and are not blocked by concentrations of agents acting selectively at receptors (58, 59). The inference usually and justifiably drawn from the actions of such generally acting substances is that a link in a common chain has been affected or that the contractile mechanism itself has been altered. We are surprised when a selective agent such as phenoxybenzamine prevents K^{+} contracture (61, 62) and this finding will be discussed below. In interpreting experiments, it is useful to consider the possible structural locations of the links in the activa-

[3] Removal of calcium from the bath medium, even if complete is not equivalent to depletion of smooth muscle calcium which often takes 30 to 90 min. before contractile ability is lost.

tion chain. Clearly the ultimate link must be in the protoplasm at the contractile proteins themselves and it is generally assumed that the proximate link, the receptors, are located at the surface membrane. This is very likely true of the striated muscle end plate acetylcholine receptors since ionophoretic injection of acetylcholine just inside the end plate membrane is ineffective, (63, 64), but for smooth muscle similar evidence does not appear to be available (46). It is of vital importance to know what links of the contractile chain of events occur in the surface membrane. Obviously our general problem is to identify and locate the links in this chain. In the summarizing figure at the end of this chapter, it will be proposed that much of the chain of activation occurs in the membrane and its inpocketings. There is not an extensive endoplasmic reticulum in smooth muscle, though so-called micropinocytotic vesicles (inpocketings) are common (65).

Although the fact does not seem to have penetrated to those who study drug-receptor interactions, smooth muscle activity also is controlled by spread of electrical activity over the cells of a smooth muscle organ, or suppression of such activity (4–7, 11, 13–15, 22, 45, 50, 51, 52, 66–77). Electrical activation and inactivation also accompanies drug-induced contraction and relaxation (4–9, 14, 17, 19, 22, 46, 50, 54). Pacemaker activity in response to mechanical strain is a phenomenon common to many but not all smooth muscles; e.g. not large arteries (4–7, 13, 14, 15, 17, 19, 22, 45, 50, 66, 67, 70, 71, 73–83), and it is conceivable that some drugs act exclusively by their effects on pacemaker cells or on the spread of electrical activity. Alternately initiation or suppression of electrical activity might be an essential intermediate step in the action of some drugs. Such drugs should have no actions when electrical activation or inactivation is impossible, e.g., in depolarized muscle. So far nearly all classes of drugs which activate or inactivate polarized smooth muscle have had similar actions on their K^+ depolarized counterparts (22, 27–39). The one exception is the cardiac glycoside group of drugs (84, 85, 86) which is known to be antagonized by K^+. Hence, most drugs would appear to activate or inactivate most smooth muscle [large arteries appear not to conduct action potentials (66, 87)] by at least two mechanisms; one involving receptors and leading to contraction or relaxation irrespective of electrical activity, and another acting via alterations in the electrical activity of smooth muscle cells. Since change in strain has itself an effect on electrical activity of smooth muscle (4, 5, 15, 19) the effect of drugs on smooth muscle electrical activity may be quite indirect, i.e., from alteration in tension via the "electrically independent" mechanism. Prevention of electrical effects by selective antagonists therefore does not provide proof that the electrical effects are directly derived from actions on receptors. In view of the unknown and complex nature of the interactions between drug effects on electrical activity and on chains of events independent of electrical activity, the development of extensive kinetic theories and polemics regarding drug receptor interactions seems a futile exercise in abstraction except in so far as such formulations allow the blocking effects of a variety of sub-

stances to be conveniently described numerically and the establishment of classes of drugs which act at the same or independent sites. It should be mentioned also that the experimental conditions often favored in studies of drug-receptor interactions (low calcium, high magnesium, low temperature, etc.) are such as to minimize initiation and spread of electrical activity. In the absence of evidence as to the nature of the accompanying electrical activities of smooth muscles studied under assay conditions, it seems desirable to reserve judgment. Use of depolarized smooth muscle in kinetic studies of drug receptor interactions would seem a logical, but so far little used experimental procedure.

In view of the foregoing complications the problems arising from studies of selective drug antagonists will be mentioned only when pertinent to other matters.

The essential role of calcium for smooth muscle contraction-interrelation to membrane Na-K transport.—The fact that depolarized smooth muscle could contract or relax when stimulated by appropriate drugs was reported in 1957 to 58 (27, 28). At that time Evans et al. (28) suggested that the mechanism might involve (*a*) a residual effect on the membrane potential; (*b*) intracellular actions of stimulants; (*c*) permeability changes unaccompanied by membrane potential changes; and (*d*) release of a substance or an ion from the cell membrane. Completely convincing evidence from microelectrode studies to eliminate the first possibility has not been presented but this possibility as well as a possible intracellular site of action, have been argued against by the availability of an adequate explanation in terms of increased Ca^{++} penetration into cells. The chief additional argument against an intracellular site of action has been the effectiveness of substances [oxytocin (28, 22), ATP (54, 83) etc.] believed not to penetrate cells as stimulants of "depolarized" smooth muscle. However, this argument rests on the pore model of the smooth muscle cell surface for which no experimental evidence exists. Arguments from the diffusion properties of stimulating substances are also not likely to be convincing in slowly responding smooth muscle systems.

The essential role of Ca^{++} in contractions of depolarized smooth muscle has been noted by a number of workers (22, 30, 31, 33, 36–39), and applies to contractions of smooth muscle irrespective of the ionic environment in which they are carried out (22). However, more recent evidence suggests that the role played by Ca^{++} is more complex (40) than a simple net influx down the electrochemical gradient as implied if the mechanism of drug action is an increase in permeability. One difficulty with this view arose early, the expected increase in efflux of $^{45}Ca^{++}$ usually could not be demonstrated on stimulation of a variety of polarized and depolarized smooth muscles (25, 32, 39, 49, 88). Another early difficulty for the simple increased permeability theory arose from further study of the finding that Sr^{+} and with less success Ba^{++} could substitute for Ca^{++} in restoring electrical and mechanical activity of polarized rat uterus and mechanical activity of depolarized uterus in response to acetylcholine (22, 40). The effects of Sr^{++} and Ba^{++} washed out much more rapidly than did those of Ca^{++}, after removal of the ion from the

Ringer solution (40), despite the similarity of their diffusion rates. In polarized intestinal smooth muscle, Sr^{++} was reported to be unable to restore electrical excitability (48), but this difference may arise from the presence of residual calcium or from a different effect of electrical and chemical stimuli on Ca^{++} or Sr^{++} permeability (40). More surprising was a subsequent report that the efflux of $^{89}Sr^{++}$ was slower than that of $^{45}Ca^{++}$ (49). These discrepancies await explanation, but the fraction of Sr^{++} or Ca^{++} bound to the site critical for contraction may be small and easily missed by conventional radioisotope procedures.

Extensive studies of the time course of the loss and recovery of responses in rat uterus have shown conclusively (40) that changes in membrane permeability to Ca^{++}, Sr^{++}, etc., are insufficient to explain the data. Release of Ca^{++}, Sr^{++} and Ba^{++} from surface binding which both increases permeability to them and increases their activity in the membrane would account for the observed results, and may be the fundamental basis for drug induced stimulation of smooth muscle (40). Potassium depolarization caused increased rate of loss of contractility in Ca^{++} free media (36, 40), probably because of decreased affinity of a cell surface site for Ca^{++}. The order of affinity of this site for the ions capable of restoring the response after Ca^{++} depletion seemed to be $Ca^{++} > Sr^{++} > Ba^{++}$ (40), based on wash-out rates and stabilization of the membrane. The well-known and widely used nonspecific direct stimulating action of Ba^{++} on smooth muscle appears to be the result of its effectiveness in activating contraction on entering the cell and of its relative lack of affinity for sites on the cell surface. The resultant failure of stabilization permits penetration of Ba^{++} through the membrane. The fact that Ba^{++} can cause activation when both Ba^{++} and Ca^{++} are present together, suggests that the concept of lesser affinity of Ba^{++} than of Ca^{++} for surface binding sites may be an over-simplification and that interactions of these two ions in a membrane transport system may be involved. When Ba^{++} is added to ordinary Ringer solutions, alterations in action potentials have been noted, consisting chiefly of the appearance of prolonged plateaus (89). The suggested explanation of these plateaus, delayed increase in K^+ conductance or decrease in Na^+ conductance, is not consistent with unstabilization by Ba^{++} unless additional postulates are made.

The complexity of the system controlling Ca^{++} entry into uterine smooth muscle of rats, cats and rabbits and its intimate relation to the system controlling Na^+ and K^+ movements has also recently been demonstrated (84, 85, 86). A close correlation has been found between actions of substances (ouabain, F^-, K^+ poor solutions) which are known to inhibit the Mg^{++} and $Na^+ + K^+$ activated membrane ATPase (90, 90a) on ion movements and on contracture.[4] When ouabain or fluoride are present in sufficient quantity to induce

[4] Copper ion is also reported to be an inhibitor of membrane Mg^{++} and $Na^+ + K^+$ activated ATPase (90) and it is of interest to note that it has been reported to be a general excitant of smooth muscle, especially when potentiated by vitamin C (91). It also inhibits net sodium extrusion (85).

downhill ion movements (K^+ loss, Na^+ gain) they also induce contractures. K^+ free solutions behave similarly except in rabbit uterus. Selective antagonists to known receptors (for epinephrine, histamine, acetylcholine, oxytocin) do not prevent these contractures. High, depolarizing K^+ concentrations prevent ouabain contracture but not fluoride and cold contractures. Low potassium media sensitized the uterus to ouabain induced contractures and downhill ion movements. Glucose free media also sensitize uteri to K^+-free contractures and ouabain contractures without increasing the downhill ion movements they produce. Cocaine (0.05 to 0.1 per cent) delays and reduces these ion movements and prevents or delays contractures. Epinephrine has similar effects, preventing or relaxing fluoride, ouabain and K^+-depletion contractures. This relaxing action was prevented by nethalide, a new beta receptor blocking agent with minimal direct sympathomimetic effects (92, 93). However unlike cocaine, epinephrine potentiated the loss of K^+, and this effect, too, was prevented by nethalide. Epinephrine also differed from cocaine in that it alone prevented cold contractures. Table I indicates the correlation that exists between apparent inhibition of membrane ATPase and contracture. Not all substances that produce downhill ion movements produce contractures, e.g. dinitrophenol (10^{-4} to 10^{-3} *M*) and iodoacetate (10^{-4} to 10^{-3} *M*) inhibit all contractions; nor do all substances that produce contracture cause downhill ion movements, e.g., cocaine in concentrations of 0.1 per cent produces profound and continuous contracture of rabbit and of cat uteri, but no K^+ loss or Na^+ gain. Not all procedures which produce contractures and downhill ion movements act by precisely the same mechanism; sudden cooling produces contracture in these uteri as well as other smooth muscle (e.g., 94, 94a) but this effect is not blocked by cocaine. The suggestion is that substances which cause downhill ion movements by inhibiting membrane Mg^{++} and $Na^+ + K^+$ activated ATPase also allow Ca^{++} entry into uterine muscle cells, or perhaps prevent its binding and extrusion, but that Ca^{++} entry can be initiated in other ways as well. Whether Ca^{++} shares the same transport system with Na^+ and K^+ (or is bound to the same sites) in smooth muscle is unknown, since an indirect effect of inhibitors of this membrane ATPase may also be involved. However, the possibility that a Ca^{++} complexing system controls contracture and relaxation of smooth muscle and is closely related to the system controlling Na^+ active transport is appealing.

Cocaine had previously been found to prevent K^+ contractures and accompanying increased ^{42}K efflux in longitudinal muscle of the intestine (38). This was reported to be a competitive relationship. Depolarization by K^+ probably inhibits active transport (95). When cocaine acts to prevent or delay contractures induced by inhibition of membrane Mg^{++} and $Na^+ + K^+$ activated ATPase, by ouabain, or fluoride, etc., it may be acting by a similar mechanism in preventing contracture by high K^+ solutions. Perhaps cocaine acts to prevent or delay the displacement of Ca^{++} from negative sites on the membrane that result from interference with the phosphorylating system in the membrane by high K^+ concentrations. Alcohol was also reported (38) to

TABLE I

Species	*Treatment*†	(*N*)	*Condition of Uterine Muscle*	*K mEq/kg Dry Wt.* s.e.
Rat	Control	5	Relaxed or Rhythmic	461 ± 32
	Ouabain $10^{-6}M$	5	Relaxed	428 ± 15
	Ouabain $10^{-4}M$	5	Slight Contracture	395 ± 14
	Ouabain $2.5\times10^{-4}M$	5	Moderate Contracture	360 ± 13
	Ouabain $10^{-3}M$	5	Complete Contracture	271 ± 7
	Ouabain $10^{-3}M$ + Cocaine (0.1%)	5	Slight Contracture	281 ± 7
	Cocaine 0.1%	5	Slight Contracture	476 ± 39
	Control	5	Relaxed or Rhythmic	473 ± 21
	NaF ($10^{-2}M$)	4	Contracture	302 ± 16
	NaF ($10^{-2}M$) + Cocaine (0.1%)	5	Partial Contracture	363 ± 20
	K free Ringer	5	Contracture	317 ± 9
	K free Ringer + Cocaine (0.1%)	5	Relaxed	364 ± 26
	Cold Ringer	5	Contracture	340 ± 13
	Cold Ringer + Cocaine (0.1%)	5	Contracture	341 ± 16
	Control	5	Relaxed or Rhythmic	416 ± 9
	Iodoacetate ($10^{-4}M$)	5	Relaxed	351 ± 25
	Iodoacetate ($10^{-4}M$) + Cocaine (0.1%)	5	Relaxed	354 ± 19
	Dinitrophenol ($10^{-4}M$)	5	Relaxed	365 ± 17
	Dinitrophenol ($10^{-4}M$) + Cocaine (0.1%)	5	Relaxed	395 ± 13
Cat	Control	6	Relaxed or Rhythmic	366 ± 20
	CHANGES FROM CONTROLS			
	Ouabain ($10^{-6}M$)	3	Contracture	−37 ± 14
	Ouabain ($10^{-5}M$)	3	Contracture	−57 ± 19
	Cocaine (0.03%)	3	Slight Contracture	−9 ± 30
	Cocaine (0.075%)	3	Moderate Contracture	+19 ± 28
	Ouabain ($10^{-6}M$) + Cocaine (0.03%)	3	Slight Contracture	−7 ± 18
	Ouabain ($10^{-6}M$) + Cocaine (0.075%)	3	Slight Contracture	+11 ± 12
	Ouabain ($10^{-5}M$) + Cocaine (0.03%)	3	Contracture	−49 ± 11
	Ouabain ($10^{-5}M$) + Cocaine (0.075%)	3	Moderate Contracture	−12 ± 24

† Uterine tissues allowed to recover for 60 min at 37°C following dissection. Control tissues then placed in fresh Krebs Ringer Solution for a further 60 min. Experimental tissues similarly treated except Ringer solution modified as indicated.

interfere with K^+ contractures, but noncompetitively. The variety of ways in which pseudocompetitive or noncompetitive interactions can occur in complex systems cautions against carrying interpretation very far at this state of knowledge.

Recently, phenoxybenzamine and SY-14(N-(2-chloroethyl-)-N-ethyl-1-naphthalenemethylamine HCl) but not yohimbine, were shown to antagonize K^+ contractures of rabbit aorta strips (61, 62). Several hundred times the amounts of these alpha receptor blocking agents required to prevent noradrenaline contractions were required to prevent K^+ contractures but the characteristics of the antagonism to K^+ as to onset, reversibility and surmountability by high concentrations were similar to those when norepinephrine was the agonist. Whether norepinephrine release is involved is not settled though the authors state that reserpinization did not affect the K^+ response or the antagonism. A question comes to mind as to whether phenoxybenzamine blocks K^+ contractures in smooth muscles which relax or are inhibited by norepinephrine. Potassium did not protect against phenoxybenzamine blockade of the norepinephrine or the potassium responses though the authors did not regard this result as conclusive. The ability of norepinephrine to protect against block of responses to K^+ was not reported. None of the data prove the hypothesis that phenoxybenzamine acts intracellularly. This hypothesis derives from an earlier study from the same laboratory of the rate of contraction to epinephrine in aorta strips at different temperatures (96). The contraction rate was assumed on tenous grounds to be diffusion controlled and the high temperature coefficient was then taken as evidence of a diffusion barrier. In fact, any step in the chain of events leading to shortening might have accounted for the high temperature coefficient. The additional data suggestive of an intracellular action of potassium are derived from the slow contraction rate relative to the onset of depolarization in taenia coli of similar dimensions. A recent report (62) states that the membrane potential changes in response to potassium are ten times faster than the contraction of pulmonary artery muscle and that SY-14 blocks the contraction but not the depolarization. Since there are several intermediate steps (probably Ca^{++} release from the cell surface and subsequent entry of Ca^{++} into the cell) interposed between depolarization and contracture, the proposal of an intracellular site of action for K^+ in these experiments seems premature. In many respects phenoxybenzamine in high concentrations appears to resemble cocaine or alcohol (38) which likewise interfere with the contractile response induced by K^+, but do not block responses to other stimulants in polarized muscle (85, 86). Furthermore, the evidence from depolarized intestinal muscle (33) that increased K^+ fluxes in response to stimulants can be dissociated from contraction by removal of Ca^{++} suggests that changes in intracellular K^+ concentration are not a sufficient mechanism for initiation of contraction. The results of Barr et al. (97) from carotid artery strips during recovery from cold storage suggest that tonic contracture to elevated external K^+ concentration is impossible until a sufficient internal K^+ concentration has been

attained but thereafter the response is related to the potassium concentration gradient rather than the internal concentration. Furthermore, with glycerol extracted uterine muscle, increases in the K^+ concentration decrease the tension developed (97a) with ATP. To say that aortic smooth muscle is different from others (61) seems an inadequate argument in view of the finding that Ca^{++} is essential for the contractile effect of K^+, as well as of other stimulants in vascular muscle (37) as in other smooth muscles (22, 36).

Mechanism of drug effects in polarized smooth muscle.—Keeping in mind that effects of drugs on polarized smooth muscle may arise more or less indirectly by effects on pacemakers and on conduction of electrical activity, it is desirable to consider the variety of responses of polarized smooth muscles to drugs. We exclude arbitrarily from consideration those drugs such as tyramine which may act wholly or in part indirectly by causing the release of other active agents (see p. 212 for a partial listing of these). That subject is in a state characterized by contradictory data and theory and could not be profitably discussed in the space and context of this review.

If the fundamental changes during contraction in smooth muscle is the release of Ca^{++} from the cell surface causing a local increase in the Ca^{++} activity and increasing its penetration through the membrane, we must consider what other permeability changes are effected by drugs in polarized muscle and how they may be related to the Ca^{++} activity of the protoplasm.

In general, the evidence as to ion movements and permeabilities of smooth muscle at rest, stimulated or inhibited is fragmentary. Only three or four muscles have been studied in sufficient detail to permit comment: these are guinea pig taenia coli (4 to 6, 14, 20, 21, 32, 33, 88, 98 to 100, 115, 115b), guinea pig longitudinal muscle of the intestine (26, 43, 44, 101), estrogen dominated rat or rabbit uterus (7–10, 12, 16 to 19, 22, 23, 24, 46, 67), and rabbit aorta (25, 39). In both taenia coli and rat uteri (20, 22, 23, 24, 99, 100, 105–107) the sodium movements studied with radioactive sodium are many times faster than the potassium movements (10 to 50×) and despite this a negative membrane potential (ca. 60 mv inside negative) is recorded with microelectrodes and net Na^+ extrusion occurs. Furthermore, the calculated sodium concentration gradient across the cell membrane is from 2 to 10 in fresh cells and the potassium concentration gradient is calculated to be 0.05 to 0.03. Unfortunately there is considerable uncertainty as to the value to be taken for the extracellular space and partly due to this but even more due to the possibility of cation exchange sites within the cell, the intracellular activities of Na^+ and K^+ may differ greatly from the calculated concentrations (20, 24, 102–106). The osmotic behaviour of frog stomach muscle (109, 110) and its permeability to large molecules while retaining K^+ (111, 112, 113) is also difficult to reconcile with the assumption that smooth muscle cells have semipermeable membranes with permeability characteristics determined by pore size and that their internal ions contribute to the osmotic pressure as expected if their activity approaches their concentration. Bozler (109–113) has considered the possibilities that cell water as well as electrolytes may be

bound and that the permeability and osmotic properties of frog stomach muscle may belong to the bulk phase rather than to the plasma membrane.

To complicate matters further, the changes in ion permeabilities on depolarization and excitation have not been fully elucidated. The coefficient relating log $[K^+]_i/[K^+]_o$ to the membrane potential has not been measured, but if $[K^+]_i$ is assumed to be constant, the coefficient relating log $[K^+]_o$ to membrane potential (outside minus inside) is from -33 to -54 (5, 6, 8, 9, 10, 14, 17, 19, 46) in a variety of smooth muscles, less than the theoretical value of about -60.5 at 37° C based on the assumptions that the membrane is permeable only to K^+ and Cl^- which are passively distributed according to a Donnan equilibrium. When K_2SO_4 rather than KCl was used to increase $[K^+]_o$ the coefficient was nearer -50 but not equivalent to the theoretical value. Parallel studies of the effect of K_2SO_4 on membrane potential in the presence and absence of sodium would be helpful. Studies of the effect of potassium ion concentration on membrane potential to date have unfortunately not given information as to cell volume, nor evidence of equilibrium so that a conclusion must be reserved. However, the suggestion is that sodium or calcium or intracellular anion movements contribute significantly to the membrane currents under the conditions of the above measurements.

When metabolic inhibitors (DNP, IAA, NaF) or ouabain (10^{-3} M was required) were used in rat uterine muscle in amounts sufficient either to cause downhill ion movements or prevent uphill ion movements, there was increased K^+ efflux and Na^+ influx in the rat uterus (22, 23, 24, 105, 106) with a smaller change in K^+ influx and no detectable change in Na^+ efflux. Removal of external potassium increased Na^+ influx and did not affect Na^+ efflux.

During activity the membrane potential falls and action potentials occur, the latter often with 10–15 millivolts reversal of membrane potential (8, 17, 19, 40, 114, 115, 115a). This appears to be less than that calculated from the Nernst equation but the intracellular Na^+ activity is in doubt (see above). Removal or reduction of external Na^+ does not rapidly decrease the velocity of depolarization or diminish the magnitude of action potentials in taenia coli (4 to 6) or pregnant cat uterus (7) as predicted if the depolarization follows a selective increase in Na^+ permeability. Velocity of conduction is also not significantly affected by lowering the external $[Na^+]$ in pregnant cat and rabbit uteri (67). However, a very recent report (115a) indicates that the velocity of depolarization is diminished in low Na^+ solutions while both the velocity of depolarization and overshoot were increased in Na^+ excess. In Na^+ free solutions the action potential amplitude was a function of the external Ca^{++} concentration. The situation may be the same in rat uterine muscle in which two investigations (16, 18) have found that the maximum velocity of depolarization or action potential amplitude is related to the external Na^+ concentration below about 60 meq/liter and another (67) has found diminished velocity of conduction of action potentials with such decreases in Na^+ concentration.

In those instances in which amplitude of action potentials was largely

independent of external Na^+ concentration, the possibility has been considered that other cations, especially Ca^{++}, might carry the depolarizing current (4, 5, 14, 45, 51, 52, 115). The ability of taenia coli to initiate action potentials in Na^+ free media depended upon the presence of external Ca^{++} (4, 115a) and removal of Ca^{++} even in the presence of Na^+ causes depolarization and electrical inactivity in several smooth muscles (4, 19, 22, 46, 51, 52). Alternately it has been proposed that the current was carried by Na^+ ions from the surface membrane which persisted in the absence of external Na^+ and were mobilized by excitation (4, 7, 18, 115). This is conceivable because the extremely rapid movement of Na^+ in taenia coli and rat uterus permit the sodium gradient to adjust rapidly to altered excellular concentrations. This explanation, if correct, would mean that the situation for sodium was essentially similar to that proposed to account for the persistence of contractile responses in the absence of interstitial Ca^{++} (see p. 193). The binding and release of Ca^{++} may depend on the same membrane transport system as that for Na^+ (p. 194) and this transport system may have a direct influence on smooth muscle membrane potential, making such results explicable.

Increased Na^+ permeability during the action potentials of smooth muscles has not been demonstrated using radioactive sodium and will be difficult to demonstrate, even in these instances in which a single stimulus leads invariably to a single fully propagated action potential, owing to the very high flux rates for this ion. Increased influx of radioactive sodium on high voltage electrical stimulation of rabbit uterus (108) is difficult to interpret since the electrical responses of the tissue have not been fully defined, much less shown to be identical to those occurring in response to chemical stimulants or strain, which are the usual stimuli for smooth muscle activity. It should be recalled that when estrogen dominated rabbit uteri were stimulated electrically or chemically shortly after exposure to a K^+ free medium, an increased net loss of potassium occurred with both types of stimuli, but an increased net gain of sodium occurred only with electrical stimuli (116). More recently a similar failure to observe increased $^{24}Na^+$ uptake was obtained on epinephrine stimulation of rabbit aorta (25).

Potassium fluxes of smooth muscle are affected by the chemical mediators, their analogs and other stimulants. Net loss of K^+ is increased from uteri during contraction initiated by a variety of chemicals (116) and $^{42}K^+$ efflux is increased while influx is decreased by a variety of contractile stimuli (cold, NaF) applied to rat uteri (105, 106). Pilocarpine- and acetylcholine-like drugs have apparently similar effects on K^+ fluxes in longitudinal intestinal muscle (25, 26, 43, 44), efflux was increased and influx decreased or much less increased. No measurement of net ion movements were made. The similarity of the effects of these autonomic stimulants on K^+ exchange to the effects of inhibitors of active transport should be kept in mind in relation to the suggestion (p. 215) that they may produce transitory inhibition of an electrogenic Na^+ pump and consequent depolarization. In this regard, the fact that carbachol increased both influx and efflux of potassium from depolarized taenia

coli (32) is of interest since depolarization usually inhibits active transport (95) so that further inhibition of active transport may have been impossible and an altered effect on potassium fluxes to be expected. Studies of polarized longitudinal intestinal muscle did not reveal consistent alterations in Na^+ and Cl^- movements, but there was an increase in Ca^{++} influx with acetylcholine-like drugs (25, 26, 43, 44). Increased K^+ efflux in this tissue in response to those drugs was obtained in this tissue after Ca^{++} depletion or cocaine had prevented contraction (43, 44, 117) and in rat uteri exposed to dinitrophenol and iodoacetate, which do not cause a contracture (105, 106). In neither case, does this prove that K^+ efflux can increase without a concomitant increase in the ability of Ca^{++} to penetrate into cells. It does show that the altered K^+ movements are not a secondary result of shortening. That increased K^+ influx and efflux and increased Na^+ and Ca^{++} influx followed carbachol in depolarized taenia coli (32) suggested that the action potentials and/or depolarization which occur in polarized muscle may be secondary to the primary effects on Ca^{++}, K^+, Na^+ or Cl^- movements. In these depolarized muscles, Ca^{++} depletion again prevented contraction but not altered ion movements (33). It must be emphasized that so far no evidence exists proving or suggesting strongly that the altered movements of K^+, Na^+, Cl^- and Ca^{++} induced by acetylcholine, carbachol and related compounds result from altered diffusion through pores. In smooth muscle, the classical types of evidence for pores [i.e., dependence of ion movement or electrical effect in ion size (117, 118); difference between the diffusion of water under osmotic and concentration gradients (119); osmotic effects of penetrating non-electrolytes of different molecular size (119)] have not been provided. It is equally or more plausible in view of Bozler's swelling and permeability data (109–113) to assume that the availability of a phosphorylated carrier or of a chain of ion exchange sites is altered. Indeed, the previous suggestion that inhibition of a membrane ATPase leads to increased K^+ efflux and Na^+ and Ca^{++} influx is just as easily understood on that basis (see 105, 106).

One enigma of polarized smooth muscle is the occurrence of periodic partial depolarizations or slow waves. These are the common electrical activity of the longitudinal muscle coat of resting small and large intestine *in vivo*. In the small intestine, these slow waves are propagated much more effectively than are the spikes which accompany contraction. They are diminished in amplitude by epinephrine and sympathetic stimulation (81) though there are contrary opinions (71) and enhanced in size leading to spikes by acetylcholine and parasympathetic stimulation (82). These and related matters have recently been extensively reviewed (14, 71).

Specific substances acting on smooth muscle. Nonselective stimulants and depressants of all smooth muscles.—Under this heading one would expect to find stimulants acting late in the chain of events which links the initial action of some drugs to contraction, i.e., in the final common pathway. *Barium*, according to an analysis of its action in polarized and depolarized uterine

muscle, should be such a substance. It substitutes for calcium as a means of initiating shortening but while displacing it from surface binding does not substitute for it as a membrane stabilizer (40). This direct action is supplemented by an indirect one on nervous tissue in some smooth muscle organs which contain intrinsic nerves (120). A recent exhaustive review of the actions of Ba^{++} (58) shows conclusively the generality of its stimulating action directly in smooth muscle, the lack of any specific or highly selective antagonists to its direct actions (papaverine is one of the better noncompetitive ones and epinephrine and reserpine are effective in tissues in which epinephrine is inhibitory) and the general ability of stimulants to show additional effects to the stimulating effects of Ba^{++}. All these generalities are to be expected according to the proposed mechanism of Ba^{++} action. The mechanism of Ba^{++} effects on nerves and striated muscles may be similar to that on smooth muscle.

A recent report (59) indicates that high concentrations ($10^{-4}M$) of morphine competitively antagonize Ba^{++} contractures in both intact intestine and nerve plexus-free circular muscle. Some other spasmolytic drugs such as 1,1-diphenyl-3-piperidinobutanol (=**I**) were additive to morphine in their antagonism to Ba^{++} contractures suggesting action at the same site. Acetylcholine responses were not affected by morphine. Papaverine-like spasmolytic drugs such as **I** are non-competitive antagonists of acetylcholine. Morphine in concentrations which prevented Ba^{++} contracture and were additive with **I** against barium prevented the noncompetitive antagonism of **I** against acetylcholine. Morphine was competitive with **I** in its interaction in acetylcholine responses. Morphine affected neither the noncompetitive papaverine antagonism to acetylcholine nor the noncompetitive antagonism of slightly higher concentrations of **I** against histamine. Interpretation of these data is difficult, but the point of great interest is the possibility that substances unrelated chemically to barium such as morphine and **I** may compete with it for a surface site.

Strontium and calcium act as universal stimulants in circumstances (such as K^+ depolarization at 37° C) which diminish the ability of these substances to combine with and stabilize the membrane and thereby diminish their ability to limit their own influx into cells (4). Elevation of potassium concentration causes contracture in all smooth muscles and can be classified as a nonselective stimulant. Its effect may be to diminish the stabilizing effect of Ca^{++} probably by decreasing the affinity of the cell surface for Ca^{++} (36, 40). That this change in affinity may be closely related to an effect on membrane ATPase has been mentioned (p. 194).

Cardiac glycosides such as ouabain may also be stimulants of all smooth muscle if the association between inhibition of "membrane carriers" and contracture recently found for several types of uteri (84 to 86) is common to all smooth muscle. Contracture of taenia coli by ouabain (121) appears to be a similar phenomenon. Other intestinal smooth muscles are also stimulated (122, 123) and K^+ efflux is increased by digitoxin (124, 125). Stimulant ef-

fects of small doses in the human uterus have been noted (126). Other inhibitors of membrane transport such as NaF (90) would be expected to have similar effects as has been observed for several types of uteri (22, 84 to 86) and other smooth muscles (e.g. 127, 128). Inhibition of "active transport" by removal of external K^+ would also be expected to cause contracture, as it does in rat and cat uteri (84 to 86, 129) and vascular muscle (97), but this does not occur with all smooth muscle (e.g., not in rabbit uteri, 84, 85, 86) owing possibly to stabilizing effects and increased membrane potential and calcium affinity from the increased potassium gradient. The contracture induced by cupric ion in a wide variety of smooth muscles (91) also may be a result of inhibition of membrane ATPase.

There appear to be some substances which can oppose to some degree the contractile effect of non-selective stimulants in a variety of tissues studied. These include above all epinephrine and related compounds, cocaine and its pharmacological relatives, papaverine-like substances and possibly progesterone. A variety of the so-called spasmolytics such as papaverine and nitrites also may fit into this category. Increasing the Mg^{++} concentration diminishes and decreasing it enhances a wide variety of contractile responses (36, see 58, 83). Lowering the Na^+ concentration sometimes increases (47, 130) but often decreases (4, 18, 22, 37, 45, 94a, 131, 132) contractile responses of smooth muscle. Finally there are the Ca^{++} complexing agents which diminish or stop contractile responses (30, 40, 83).

Epinephrine.—The inhibiting or relaxant effects of epinephrine and certain related amines appear to be universal in that they are obtained in all smooth muscles and oppose all stimulants provided that contractile responses to epinephrine in those tissues which possess them (see p. 210) have first been inhibited by phenoxybenzamine or another selective "alpha" blocking agent. Alpha blockade serves to unmask epinephrine relaxing or contraction-preventing effects in all tissues in which contractile effects are predominant and in which this has been investigated 133–137). This unmasking technique which allows the excitatory and inhibitory effects of epinephrine and like substances to be studied in the same preparation has not been sufficiently utilized in attempts to delineate the mechanisms of excitatory and inhibitory actions of epinephrine on smooth muscle. Inhibitory and excitatory actions of epinephrine cannot be casually identified with beta and alpha receptors, since these are defined by the selectivity of the tissues toward agonists and antagonists rather than by the nature of the response (133, 134). Recently intestinal muscle has been found to contain both alpha and beta receptors so defined, both of which caused inhibitory effects (138, 139, 140).

Conversion of an inhibitory response to an excitatory response in an isolated smooth muscle by block of "beta receptors" has some possible theoretical significance (see below) and has been reported infrequently [e.g., in prolonged experiments (141–143)] possibly owing to the lack of beta receptor antagonists which were not also agonists (144, 145). A new agent, nethalide (92, 93), appears to offer much more selectivity in this regard, but few studies using it have so far been reported. A water extractable material from uteri of

pregnant and progesterone treated cats has been demonstrated to convert the the response to epinephrine of uteri from estrus cats from inhibition to excitation (146). The conversion of response by this material (receptors in a test tube?) was prevented by phenoxybenzamine. The exciting possibilities opened up by this finding have not so far been pursued.

Conversion of an epinephrine inhibitory to an excitatory response has been achieved in taenia coli by depletion of glycogen in a glucose free medium, by inhibition of glycolysis with iodoacetate (4, 45, 79, 144, 148). The hypothesis was put forward that the increased metabolism in response to epinephrine normally stabilized the membrane, or increased the activity of an electrogenic Na^+ pump, overcoming the concomitant excitatory effect. Inhibition of the metabolic response in a variety of ways then unmasked the excitatory effect.

In mesenteric arteries (149), contraction in response to epinephrine and other stimulants (acetylcholine, histamine, Ba^{++} and high voltage a.c. electrical current) was accompanied by increased glycolysis and lactate production under both aerobic and anaerobic conditions. Contractions were decreased under anaerobic conditions by glucose depletion and by iodoacetate, NaF, and dinitrophenol when these were added in the extraordinarily large amounts ($\geq 10^{-2} M$) which were found necessary to interfere with the increased metabolism on stimulation. Considerably smaller amounts of iodoacetate and dinitrophenol inhibited contractures of uteri (22, 85). Increased metabolism of glycogen or glucose to lactate on stimulation did not markedly increase ATP or CP levels in arterial muscle but was calculated to produce more energy rich phosphates than were utilized for external work, or for shortening or recovery (150).Consequently about 80 per cent of the extra energy available appears to have been consumed during contraction chiefly for the activation process. Energy stores were so small as to require the utilization of metabolic energy for contraction or rather for activation (147). In an earlier study (165, p. 318) these authors proposed that epinephrine may "by separate mechanisms, activate either the carbohydrate metabolism or both this and the contractile mechanism." The question must be posed as to how it was possible for interference with the epinephrine induced increase in metabolism in taenia coli to convert inhibition to excitation if excitation itself requires increased metabolism. If the "activation of carbohydrate metabolism" by an independent mechanism provides the energy needed during contraction, then prevention of that response by an agent such as nethalide should prevent the contractile response as well and conversion of an inhibitory to an excitatory response in this way should be impossible. Clearly, studies of the effects of nethalide and related substances on the contractile and metabolic responses to epinephrine of tissues such as taenia coli and of the effects of agents such as nethalide and of phenoxybenzamine on tissues such as mesenteric arteries and rabbit uterus might help elucidate these problems.

In any case, epinephrine relaxing effects appear to be accompanied by increased metabolism, increased tissue levels of energy rich compounds,

increased membrane polarization (4) and possibly by an increased rate of Na^+ extrusion. The membrane effects were at first thought to be correlated with conversion of phosphorylase b to a (4, 148). More recently increase in the levels of ATP+CP has been found to precede conversion of phosphorylase b to a as does too the relaxant effect (151). Such results exclude theories that suggest a depletion of energy rich phosphates as a mechanism of the inhibitory action of epinephrine (152).

The estrogen-dominated rat uterus is also relaxed by epinephrine, but studies *in vivo* following intraperitoneal injection of epinephrine failed to show conversion of phosphorylase b to a (153) despite decreased glycogen levels. The associated vasoconstriction and anoxia may have complicated the result. Earlier studies showed decreased ATP+CP levels under these circumstances (154, 155). *In vitro* studies (156) suggest that the activation of phosphorylase (e.g., by EDTA) in this tissue may be unusual. In tracheal smooth muscle, as in taenia coli, phosphorylase activation was produced by epinephrine, but this effect was not considered to be causally related to relaxation (157). Epinephrine did not cause activation of phosphorylase in arterial muscle which it contracted (157).

Lactate production, glycolysis and tissue lactate concentrations are also increased during the relaxant action of epinephrine and there has been an extensive series of papers over 10 years (127, 128, 159–165) supporting the hypothesis that lactate accumulation is the immediate cause of inhibitory effects of epinephrine on smooth muscle. These same authors have now reported (149, 150) as mentioned above that the contractile effects of epinephrine and a variety of other substances are accompanied by increased glycolysis and lactate production. Many of the data are presented without sufficient explanation (see e.g., 149, Figure 1 and 2) so that, detailed analysis is difficult. No discussion as to the relation of these findings to the lactate theory is given. A previous publication (165) suggests that the increased lactate production interferes with the epinephrine contraction, partly depressing it. If so, then inhibitors of glycolysis and lactate production might have potentiated epinephrine contractions, but instead epinephrine contractions were diminished. Most of the early objections to the lactate theory (see 166) were countered at least in part (164, 165), but the theory has not been proved or accepted. A recent discussion presents a variety of new evidence against any role for lactate in functional vasodilation (167) in man. Obvious experiments such as the effects of alpha and beta blocking agents on contraction and lactate production in various tissues with either excitatory and inhibitory primary responses to epinephrine, the effects of epinephrine on lactate production during relaxation of depolarized smooth muscle, the effects of epinephrine on lactate production when relaxation is induced by action at alpha receptors of intestinal muscle have not been carried out. An experiment in which relaxation occurred without lactate production or with decreased lactate production will be required to demolish this theory since experiments in which lactate production without relaxation occurs do not suffice. Epinephrine relaxa-

tion is not simply cessation of active contraction (see 168) so its relation to metabolism is of central importance.

The recent finding (85, 86) that brief (1 to 2 min) removal of glucose potentiates contractile responses to ouabain and K free Ringer solution in rat uterus while epinephrine inhibits such responses in concentrations (1 μg /ml) which do not markedly inhibit acetylcholine contractions, is undoubtedly related to the above phenomena. Glucose transport through the smooth muscle cell membrane may be via a membrane carrier system which in turn is under the control of epinephrine. The glucose carrier system might be interrelated in some way to the Na and K transport systems so that contracture or inhibition of the latter by ouabain, K free Ringer solution etc. is prevented by epinephrine.

Epinephrine itself prevents contractures induced by inhibitors of membrane Mg and Na+K activated ATPase in rat uterus but enhances the loss of potassium and gain of sodium which they induce. This enhancement along with the relaxation is prevented by nethalide (85, 86), and could not be reproduced by 3′,5′ cyclic AMP. Reversal of contracture without reversal of downhill ion movements by epinephrine along with its relaxant action in depolarized muscle (29) suggests that the primary relaxant action of epinephrine is in the membrane via an action on Ca^{++} complexing and that metabolic effects including those of the Na-pump are secondary. In taenia coli, epinephrine has been stated to accelerate sodium extrusion (4), but these studies were presumably done in the absence of inhibition of membrane ATPase.

Spasmolytic drugs.—Spasmolysis is here used to indicate antagonism to a wide variety of contractile substances in a wide variety of smooth muscles. The chain of events initiated by such substances probably interacts with the chain leading to contraction in that part which is common to all stimulants. Papaverine and the nitrites are the commonly mentioned examples and, epinephrine and related amines either alone or after alpha receptor blockade should perhaps be placed in that category, despite the fact that their relaxant effects can be selectively prevented. This fact has no bearing on the place of interaction of epinephrine effects with the contractile process. A suggestion worthy of experimental examination is that all such substances act at a step in the final common path to contraction probably by preventing calcium release from its complexes at the surface membrane. So far, nothing is known of the effects of spasmolytic drugs on ion movements between smooth muscle cells and their environment.

Cocaine.—Cocaine has been found to depress the contractile response of Ca^{++}-depleted, depolarized intestinal muscle which occurs on restoration of Ca^{++} (38, 44, 117). It has also been found to prevent the contractures provoked in rat and cat uterus by inhibitors of membrane ATPase (ouabain, NaF, K^+ free media) and this is associated with decreased downhill movements of Na^+ and K^+ across uterine cell membranes (85, 86). Since K^+ contractures are also believed associated with inhibition of active ion transport, there may be a common mechanism underlying these experiments. Cocaine

may prevent or delay dissociation of Ca^{++} from surface sites which ordinarily occurs on inhibition of membrane ATPase. The question arises, do other drugs such as acetylcholine act similarly, i.e. by transitory inhibition of membrane ATPase. No final answer can be given, but doses of cocaine and epinephrine which prevent ouabain, NaF and K^+ free contractures do not fully block responses to acetylcholine and ATP.

Both cocaine and procaine in slightly higher concentrations than those used to block contractures provoke a contracture which lasts many hours, which is not accompanied by downhill ion movements. Contractures were not provoked by inhibitors such as dinitrophenol and iodoacetate which provoked downhill ion movements. Inhibition of membrane ATPase but not generalized inhibition of metabolism was a sufficient though not a necessary condition for contracture (85, 86). It is unlikely that epinephrine and cocaine are acting by identical mechanisms in polarized muscle since they have different effects on downhill ion movements. Another difference between epinephrine and cocaine is that only the former can prevent cold contracture. The possibility that both cocaine and epinephrine as well as phenoxybenzamine (p. 196) and alcohol (p. 194 and 196) in high concentrations share a common effect in depolarized muscle must be considered.

Ethylene diamine tetraacetic acid (Na_2EDTA or Na_4EDTA) will also probably relax all contractures of smooth muscle. There is usually a preliminary brief contracture. The actions of EDTA are not equivalent to withdrawal of Ca^{++} from the medium (see below) but rather result from Ca^{++} depletion from the cell surface (40, 83). $MgNa_2EDTA$, but not $CaNa_2EDTA$, is an effective inhibitor (83). No evidence of Ca^{++} complexing by epinephrine or cocaine exists. Large increases in Ca^{++} activity are required to antagonize cocaine inhibition (38) and epinephrine (58). Cocaine, epinephrine etc., therefore, act differently from EDTA in preventing or relaxing smooth muscle contractures.

Progesterone.—A substantial body of indirect evidence has accumulated (8, 9, 19, 74–77, 169–173) indicating that progesterone inhibits uterine muscle responsiveness, especially to oxytocin and stretch. When estrogen is injected into rats or rabbits the membrane potentials of uterine cells increase (8, 9, 10, 12, 19, 77) as does their excitability. Progesterone injection increases the membrane potential further (8, 9, 19, 77), and diminishes excitability (74–77, 169–173) in response to stretch and oxytocin. It also interferes with propagation of action potentials (8, 67, 75, 77). The potassium gradient is unchanged or increased (75, 174) and the rate of loss of responses in low calcium solutions is decreased (42), leading to the suggestion of increased affinity of Ca^{++} for the cell surface after progesterone. Oxytocin sensitivity of the uterine muscle in a variety of animals (74–77, 169–172, 175–178) and in humans (178–181) may be related to the relative proportions of progesterone and estrogen acting on the muscle (182) (see p. 211). However, the effectiveness of progesterone in threatened premature labour is not established (183). In some species (e.g. guinea pig) progesterone fails to inhibit or even enhances uterine contractions *in vitro* (184, 185). A number of adrenal

steroids potentiate arterial muscle contractions (186, 187, 187a). Recently inhibition by progesterone and some other steroids of contractions of a wide variety of smooth muscles as well as uterus has been demonstrated *in vitro* (188–191). Perhaps progesterone will be placed in the same class of depressant as cocaine on further study. A possible antagonism between the cardiac glycosides and progesterone-like steroids (191) is of considerable theoretical interest.

Stimulants of isolated smooth muscle; acetylcholine.—Although acetylcholine and related compounds relax most blood vessels in the intact organism and are said to act similarly on certain sphincters (e.g., bladder), few published instances of such an action in an isolated smooth muscle are known to the author. Instead acetylcholine usually contracts or does not affect such preparations (p. 213). This enigma has perhaps become so commonplace as to be ignored but still awaits solution.

The stimulant action of acetylcholine and related compounds results in increased K^+ efflux and an increased calcium influx (but not efflux) (p. 192). Depolarization and increased production of action potentials are found in polarized smooth muscle (p. 191) but these events are not necessary for contraction since permeability changes and contraction occur in the absence of membrane potential changes in depolarized muscle. Furthermore, the contraction fails to occur even though altered potassium fluxes do occur if calcium depletion has been carried out (p. 200). Depletion and restoration of calcium in the continued presence of acetylcholine result in relaxation and contraction of "polarized" uterine muscle but do not affect tone if acetylcholine is absent (40) and repeated stimulation with acetylcholine increases the rate of decline of responses in calcium free media (36), all indicating that acetylcholine mobilizes surface Ca^{++}, increasing both Ca^{++} activity in the membrane and leading to penetration of the membrane by Ca^{++} as well as loss to the interstitial fluid. It is hard to understand why loss of ^{45}Ca is not increased to some degree in such circumstances (p. 192) and it may be that its occurrence will be noted when experimental conditions are properly arranged to detect loss from a small fraction of the Ca^{++}. Much remains to be determined, e.g., how acetylcholine combination with a receptor alters affinity of a binding site and initiates calcium mobilization, but the next few years should yield many advances.

How acetylcholine produces relaxation of some of the same smooth muscles *in vivo*, and apparently by combining with the same receptors (since atropine block both responses) cannot be answered. If experimental conditions could be arranged for production of relaxation of acetylcholine by isolated smooth muscle this would be an initial step toward finding an answer.

Serotonin.—Serotonin also appears to contract most but not all types of isolated smooth muscle (192–196). Woolley (197) has proposed that Ca^{++}-serotonin complexes with membrane lipid carriers are formed which enable Ca^{++} to penetrate the cell membrane. At the inner cell surface, such complexes are believed to dissociate into Ca^{++} and serotonin as a result of an alteration of the carrier molecule. A variety of carriers, each specific for one of

the groups of chemical stimulants, was believed to account for smooth muscle stimulation. Research following up this stimulating idea has so far yielded a number of possible lipid carriers but they lack the desired specificity, working also for norepinephrine (198, 199). Born (196) has shown that taenia coli take up labelled serotonin in excess of that attributable to extracellular fluid, but that most of the intracellular serotonin has been degraded by monoamine oxidase. Whether the intact serotonin molecules found in the tissue and not attributable to interstitial fluid after inhibition of the oxidase were actually free inside the cell or bound at the cell surface could not be determined. The temperature dependence of serotonin uptake suggested a diffusion rather than a membrane carrier process. In a study of membrane effects during development of tachyphylaxis toward acetylcholine, histamine, and serotonin (80), only serotonin showed consistent and prolonged reductions of tension and electrical response. Its effect on the delay between addition of drug and contraction was also more marked and it alone produced marked tachyphylaxis on repeated administration of the samedose, not requiring interposition of a large dose as did histamine and acetylcholine. These differences were suggested to be related to the cellular uptake or binding of serotonin (196).

Studies of the contractile effects of serotonin on the cat spleen (194) have suggested that most of these result from norepinephrine release but that serotonin has some direct action on adrenergic receptors. This mechanism cannot of course account for its contractile action on rat uterus, intestinal muscle and other tissues which are relaxed by norepinephrine, but phenoxybenzamine is an antagonist of these muscle effects (192, 193). Serotonin relaxes the frog lung smooth muscle and the relaxation is not affected by known antagonists; though agents preventing epinephrine-like relaxing actions were not tried (195). Hence, in this instance serotonin may be acting indirectly as in the cat spleen. The action of serotonin on vascular smooth muscle *in vivo* also appears to be indirect in part and remains a puzzle (200).

ATP, ADP etc.—Adenosinetriphosphate is another substance which appears to be chiefly contractile in its effects on isolated smooth muscle (54, 83, 201, 202, 203) but is a relaxant *in vivo*, especially on the vascular system (167, 204, 205, 206). ATP and ADP were about equipotent as vasodilators in the rat when trapping of ATP in transit through the lungs was avoided by intra-arterial injections and were over 100 times more potent than AMP (204, 205). ATP and ADP appear not to play a role in functional vasodilation since they are not produced by contracting muscle and probably do not pass the cell membrane (167).

In isolated rat uterus, ATP and ADP, but not AMP, adenosine or adenylic acid cause contraction in concentrations ranging from $10^{-6}M$ at 37° C to $5\times10^{-3}M$ at 24° C in both polarized and depolarized muscle (54, 83, 201, 202). Action potentials accompanied contractions in polarized muscle (54, 83). CTP and CDP have similar effects (83) though there is some disagreement on this point (202). UTP and GTP have not been adequately tested. Contractile responses to these nucleotides were not prevented by antagonists

to acetylcholine, serotonin, histamine and oxytocin (83). They were prevented or decreased by cocaine (0.1 per cent) but epinephrine (1 to 5 μgm /ml) did not relax them completely. ATP contractions were enhanced by brief removal of Mg^{++} or a brief reduction of Na^{+} or H^{+} concentration but were reduced by brief removal of Ca^{++}. Prolonged reduction of the medium Ca^{++} concentration prevented contractile responses to ATP as to other stimuli. Brief reduction of K^{+} concentration or removal of glucose in the medium did not enhance ATP contractions though either change enhanced acetylcholine contractions, but both changes simultaneously enhanced both acetylcholine and ATP contractions. Ouabain in amounts slightly less ($10^{-4}M$) than were required to inhibit active transport in rat uterus did not potentiate or suppress ATP contractions. Metabolic inhibitors (DNP or IAA) suppressed ATP contractions in parallel to suppression of contractions by acetylcholine. ATP and ADP are known to have the ability to complex Mg^{++} and to a lesser extent Ca^{++} (202a). The above and additional data were therefore interpreted to suggest an action at the cell surface to complex Mg^{++} allowing Ca^{++} to occupy a carrier for which the two cations compete (36) thereby promoting Ca^{++} entry into muscle cells and contraction. Whether this carrier was part of a membrane ATPase was not decided by the data, but seems unlikely. Tests with other agents able to complex Mg or Ca to differing degrees supported this hypothesis and suggested the value of the concept that the cell surface contains complexing sites for alkaline earth cations. Some of its properties may be elucidated by studies of its interactions with a variety of complexing agents (83).

ATP (1–5 mM) also contracted isolated glycerol-extracted arterial and uterine muscle but this was via a different mechanism since removal of Mg^{++} diminished the response and lowering the pH below 7 to 6.5 potentiated it 97a, 203). ADP was ineffective in contracting glycerinated uterine muscle as were oxytocin, epinephrine and acetylcholine (97a). Contradictory statements as to the effect of ATP and ADP on unextracted arterial muscle have been made, both contraction and relaxation having been reported [see disc., (203)].

Histamine.—Histamine is like acetylcholine and ATP in having relaxant actions in most blood vessels *in vivo* and contractile actions *in vitro* (60, 207). As with acetylcholine and ATP no satisfactory explanation is available for the discrepancy. Larger amounts are required in all three cases for the contractile effect (167, 207). An indirect action *in vivo* may be involved, but no satisfactory candidate for the effective relaxing substance *in vivo* exists. Perhaps a recent hypothesis suggested by Hilton to explain functional vasodilation provides a way out of the difficulty (167). Relaxation is supposed to be propagated along the blood vessels *in vivo*. If this is correct, the problem becomes to discover what structures *in vivo* are stimulated to set up propagated relaxation in chemically induced vasodilation and the mechanism of propagation.

Histamine differs from acetylcholine and possibly from ATP in having no direct stimulating action on a variety of smooth muscles *in vivo*. Rat uterus

which is also inhibited by epinephrine and papaverine has been the common example but recent results complicate the picture. Histamine alone has an inhibitory effect on contractions in response to other substances. Histamine, but not papaverine also diminishes the inhibitory effect of adrenaline suggesting that histamine acts on the same structure or on a subsequent stage of the epinephrine initiated inhibition process (208). Furthermore, histamine sometimes became stimulating in prolonged experiments on rat uterus, either causing contraction or potentiating contractions from other agonists (143). This reversal of effect was facilitated by dichloroisopropylnorepinephrine which also reversed the epinephrine inhibition to excitation in some experiments. Papaverine inhibition was never reversed. However, reversal of histamine effects was not closely linked to reversal of epinephrine effects and once reversed, epinephrine effects were blocked selectively by phenoxybenzamine and reversed histamine effects were selectively blocked by mepyramine (143). The selectivity of phenoxybenzamine toward epinephrine is somewhat surprising in view of its ability to prevent histamine contractions in other smooth muscles (209).

The finding (210) that histamine actions on the isolated stomach are indirect, via release of acetylcholine and norepinephrine, impels caution in interpretation of data regarding histamine actions in smooth muscle until independent direct action has been demonstrated. There is however no doubt that histamine has direct contractile effects on many types of uterine muscle other than from rat (see 211, p. 648 and p. 656) and on some intestinal muscles. The nature of the electrical and permeability changes associated with histamine contractions have not been thoroughly studied but potassium loss from cat and rabbit uterus *in vitro* (116) was accelerated by histamine and a loss of potassium *in vivo* chiefly from intestinal muscle appears to account for the increased plasma potassium concentration (212). Model experiments have been reported which support a hypothesis that histamine interacts with primary phosphate esters at the cell surface and that this effect is antagonized by calcium and antihistamines (213). Belleau (214) has proposed that adrenergic drugs which combine with alpha receptors interact by electrostatic attraction with a surface phosphate anion, suggesting a chemical basis for certain similarities between the actions of histamine and epinephrine.

Norepinephrine, epinephrine as stimulants.—In contrast to their general ability to inhibit smooth muscle, norepinephrine, epinephrine and related compounds initiate contraction in relatively few smooth muscles (215–219). The best studied electrophysiologically is the guinea pig vas deferens (215–217). This muscle is not spontaneously active and responds to stimulation of the hypogastric nerve by the production of junction potentials which sum if stimulation is repeated to yield a spike. Spontaneous release of mediator, probably norepinephrine (216) occurred, producing miniature potential changes. These were affected similarly to spontaneous end plate potentials by a variety of experimental variables (14, 217). Similar effects of sympathetic nerve stimulation have been obtained with a number of other

preparations (218, 219). So far analysis of the permeability changes underlying norepinephrine depolarization of these smooth muscle has not been reported, but the availability of appropriate techniques now makes such results readily obtainable. Previous work (25, 116) using other methods suggests that an increase in K^+ permeability occurs in uterine and aortic muscle. In aorta only the increased Ca^{++} influx was correlated with contraction. In neither instance was the uptake of Na^+ increased by epinephrine.

Oxytocin.—Having begun with the least selective, we arrive last at the most selective of the smooth muscle stimulants, bypassing many en route. The oxytocins act almost exclusively on uterine muscles and myoepithelial cells of the mammary gland (see 220). In estrogen-sensitized rats, chickens, or dogs, oxytocin causes vasoconstriction (see 220a); chemical or surgical sympathectomy also results in the appearance of vasoconstriction to oxytocin. The actions of oxytocin on the uterus had not been selectively blocked until recently (221, 222). Elucidation of the structures (223, 224) and synthesis of oxytocins and analogs (225–229) have been a stimulus to study of oxytocin structure-activity relations. Some of the analogs closely related in structure to the oxytocins (221) as well as substances of much simpler structure (such as alpha thioglycerol (222)) have been found to prevent oxytocin activation competitively. Similarly, study of the various oxytocin analogs has revealed structural requirements for activity as well as block (221, 225–229). Hence, the criteria for the existence of specific receptors have been fulfilled. But how does oxytocin exert its stimulant effect? Oxytocin like every other stimulant of smooth muscle except cardiac glycosides produces a contraction of depolarized as well as polarized muscle (28) but requires Ca^{++} in the medium for its effect whether the muscle was polarized or not (22). In normal Krebs medium oxytocin depolarizes and initiates action potentials; (19, 77, 114) in a calcium-poor medium the partly depolarized muscle can be polarized by oxytocin to a level compatible with action potentials (46). Many studies attest that progesterone *in vitro* as *in vivo* diminishes responses to oxytocin in rabbit uterus and in a variety of other uteri. The mechanism of this effect is unknown (see p. 206) but may involve a change in the affinity of surface calcium after progesterone treatment. Another recent and interesting finding is that in dogs, though not in other species studied (230), synthetic oxytocin raised the blood sugar by what appeared to be a direct effect with possible physiological significance. In puerperal women, however, it lowered blood sugar (231).

Other "oxytocics."—To apply the single term oxytocic to all substances which contract the uterus seems to be a confusion of terminology. Innes (232) has recently shown that the ergot alkaloids, which have been widely used as "oxytocics," are in fact acting on epinephrine receptors and probably not on oxytocin receptors at all. The general applicability of his finding for ergot alkaloids requires further study, but histamine and epinephrine acting on the uterus are certainly acting at their own independent receptor sites. Hence, the reservation of the name oxytocic for those substances which act at the

TABLE II

Releaser	*Organ*	*Released*	*Effect*	*Reference*
Histamine	Stomach	Acetylcholine Norepinephrine	Contraction Relaxation	210
Relaxin	Rat Uterus	Epinephrine	Relaxation	233, 234
Morphine*	G. pig Intestine etc.	Decrease Acetylcholine Increase Norepinephrine	Inhibits	235, 236, 237
Angiotensin*	Rabbit and G. pig Intestine	Acetylcholine	Contraction	238, 239
Darmstuff*	G. pig Intestine	Acetylcholine	Contraction	240, 241
Barium ion*	Intestine etc.	Acetylcholine	Contraction	120
Gluten	G. pig	Decreases Acetylcholine	Inhibits	242
Catechol	Intestine	Acetylcholine	Contraction	243

* Also has direct effect.

same receptors as oxytocin might clarify our concepts and terminology in this field. The possible objection that oxytocic is derived from oxytocia meaning "hastening childbirth" is not a serious obstacle since no other uterine stimulant than oxytocin can hasten childbirth unless promoting abortion is considered hastening childbirth.

Indirectly acting substances.—For the purposes of this review, substances acting indirectly on smooth muscle via the release of mediators or other active substance are important only in that they should be identified and eliminated from consideration as acting on smooth muscle.

Fortunately, the means of eliminating such substances (other than denervation) are becoming available (reserpine, guanethidine, etc., for those acting via release of catecholamines; hemicholinium and related compounds for those releasing acetylcholine; reserpine for those releasing serotonin; compound 48–80 and others for histamine releasers). Unfortunately, the use of these tools is revealing an unexpectedly large number of indirectly acting substances and the list is no doubt far from complete. Some of the more recent unusual or interesting additions are listed below. Indirect effects of tyramine, guanethidine and serotonin and related compounds are considered too well known for documentation. One interesting finding is that epinephrine and norepinephrine levels are under hormonal control in the rat uterus (233, 234),

estrogen increasing and progesterone decreasing these catecholamines. Relaxin inhibitory effects are brought about by release of epinephrine with the threshold to relaxin related inversely to the concentration of uterine epinephrine. Spontaneous contractions were also inversely related to epinephrine content. (See Table II.)

It behooves all those who study drug effects on smooth muscle to consider possible indirect effects at an early stage of the investigation of compounds with an unknown mechanism of action. A number of the instances in which an outstanding discrepancy between *in vivo* and *in vitro* effects remains a mystery should be reinvestigated to rule out indirect effects: e.g., acetylcholine on blood vessels, histamine on blood vessels, serotonin on blood vessels, morphine on dog intestine etc.

Sphincters.—One of the persistent myths of pharmacology is that all the so-called sphincters respond oppositely from the remainder of the viscera to chemical mediators (211, p. 395). For example in the intestinal tract which usually contracts in response to parasympathomimetic stimulation and relaxes in response to sympathetic, one is told that the cardiac sphincter, the pylorus [not really a sphincter (71)], the ileo-colic sphincter and internal anal sphincters contract to sympathetic stimulation and relax to parasympathetic. The evidence on which this mythology arose is not easily discovered and recently contrary evidence has become available, though it is doubtful that such a didactically satisfying myth will perish readily. Some of this recent evidence is tabulated as follows (see Table III).

Summary

The evidence considered in this review and the conclusions drawn can be unified in part from the point of view mentioned in the initial sections. An attempt has been made to diagram the interrelations between classes of drugs which have been suggested (Fig. 1). The diagram is divided into extracellular fluid, cell membrane, and cytoplasm. Central to the working and unifying hypothesis which the diagram is intended to represent is the concept that the affinity of membrane anionic sites for calcium ultimately control contraction of smooth muscle. In addition, functioning of membrane ATPase and consequently of an electrogenic ion pump is believed to play a major role in determining the affinity of these anionic sites for calcium. Inhibition of this ATPase by cardiac glycoside, F^-, and K^+ depletion is postulated to act directly or indirectly to diminish the affinity of membrane sites for calcium leading to depolarization, calcium release and entry and contracture. One of the major questions to be answered by future research is whether various stimulants or inhibitors of contraction act directly to alter the affinity of anionic membrane sites for calcium or through inhibition of the ion pump. The interrelation between the electrogenic ion pump, affinity of anionic sites for calcium and calcium release is suggested by enclosing them together in a box. That there is an unanswered question as to how various stimulants and inhibitors work is suggested in some cases by arrows pointing to both inhibition of the ion pump and altered affinity of surface sites, thin arrows for en-

TABLE III

Sphincter	*Preparation*	*Responses*	*Receptors*	*References*
Cardiac Sphincter	Vagi cut, Rabbit *in situ*	Sympathomimetics* Relax	α and β	(140)
	Man; other species	Parasympathomimetics* Contract	Muscarinic	(244)
Pyloric "Sphincter"**	G. pig, kitten, rat, mouse Isolated	Parasympathomimetics Contract	Muscarinic	(210)
		Sympathomimetics Relax?	?	
	Dog *in situ*	Parasympathomimetics Contract	Muscarinic	(245)
		Sympathomimetics Inhibit	β and perhaps α	
	Cat Isolated	Parasympathomimetics Contract	Muscarinic	(245)
		Sympathomimetic Inhibit	β and perhaps α	
Sphincter of Oddi	Calves or cat Isolated	Parasympathomimetics Contract	Muscarinic	(246 to 248)
		Sympathomimetics Contract		
Ileo-Colic "Sphincter"**	Rabbit Isolated	Parasympathomimetics Contract		(249)
		Sympathomimetics Inhibit		

* Norepinephrine and epinephrine are considered sympathomimetic and acetylcholine, carbachol, pilocarpine etc. are considered parasympathomimetic.

** These may not be sphincters in the sense that a pressure drop across them has not been reported.

hancing effects and thick arrows for suppressing effects. High potassium and low chloride probably act at one or both of these sites. Epinephrine opposes contractures by combining with beta receptors; possibly leading to stimulation of the ion pump by increasing metabolism or by acting directly on the pump. On the other hand, when membrane ATPase is inhibited epinephrine still inhibits contractures but does not oppose downhill ion movements. Therefore, it may act to affect affinity of surface sites for calcium either as a primary or as a second independent mechanism. The possibility that barium inhibits uphill ion transport is not excluded though no arrow was drawn. Sodium EDTA is known to inhibit active ion transport, and even depolarization of the surface membrane by action potentials or strain may effect uphill ion transport. In these cases, it appears likely that there is no direct effect on the ion pump and changes in uphill transport are secondary consequences of the removal or displacement of calcium from surface sites. Arrows were drawn only to indicate supposed direct effects.

For drugs which are known to act on receptors, as defined by agonist and antagonist selectivity, combination with receptors at the cell surface has been indicated as an intermediate step. For stimulant drugs acting at receptors no arrow has been drawn to suggest inhibition of the ion pump, though a transitory direct effect of this sort has not been entirely excluded. Such drugs have an accessory or dual mechanism of action in another sense since they can induce action potentials either by their tension increasing effect (illustrated) or as a result of calcium release and altered ion permeability (not illustrated). However, depolarization and action potentials are not essential for the contractile effects of these drugs.

ATP and ADP are most likely producing contractile effects by tying up membrane magnesium so that it cannot compete with calcium for carriers or exchange sites. A possible effect to inhibit the ion pump by interfering with membrane ATPase has not been excluded. Na_4EDTA and Na_2EDTA initiate submaximal contractions and the mechanism also seems to involve complexing of membrane magnesium, though unstabilization by complexing of superficial calcium may facilitate entrance of carrier bound calcium. EDTA contractions rapidly relax and inhibition of all contractile responses follows owing to complexing of the membrane calcium required for subsequent contraction. The mechanism whereby very small concentrations of ATP and ADP relax blood vessels *in vivo* is unknown.

Cocaine opposes the consequences (contracture and downhill ion movements) of application of inhibitors of membrane ATPase. It is less effective against stimulant drugs acting at receptors. Therefore, its site of action may be closer to the membrane ATPase system than to the sites of calcium binding. Progesterone-like steroids are suggested to have a similar site of action because they increase membrane potential and may oppose the actions of cardiac glycoside steroids while decreasing contractility. Phenoxybenzamine and alcohol may act at a similar site. Papaverine-like spasmolytics seem more likely to act at surface sites for calcium complexing than at the pump mechanism but no evidence is available.

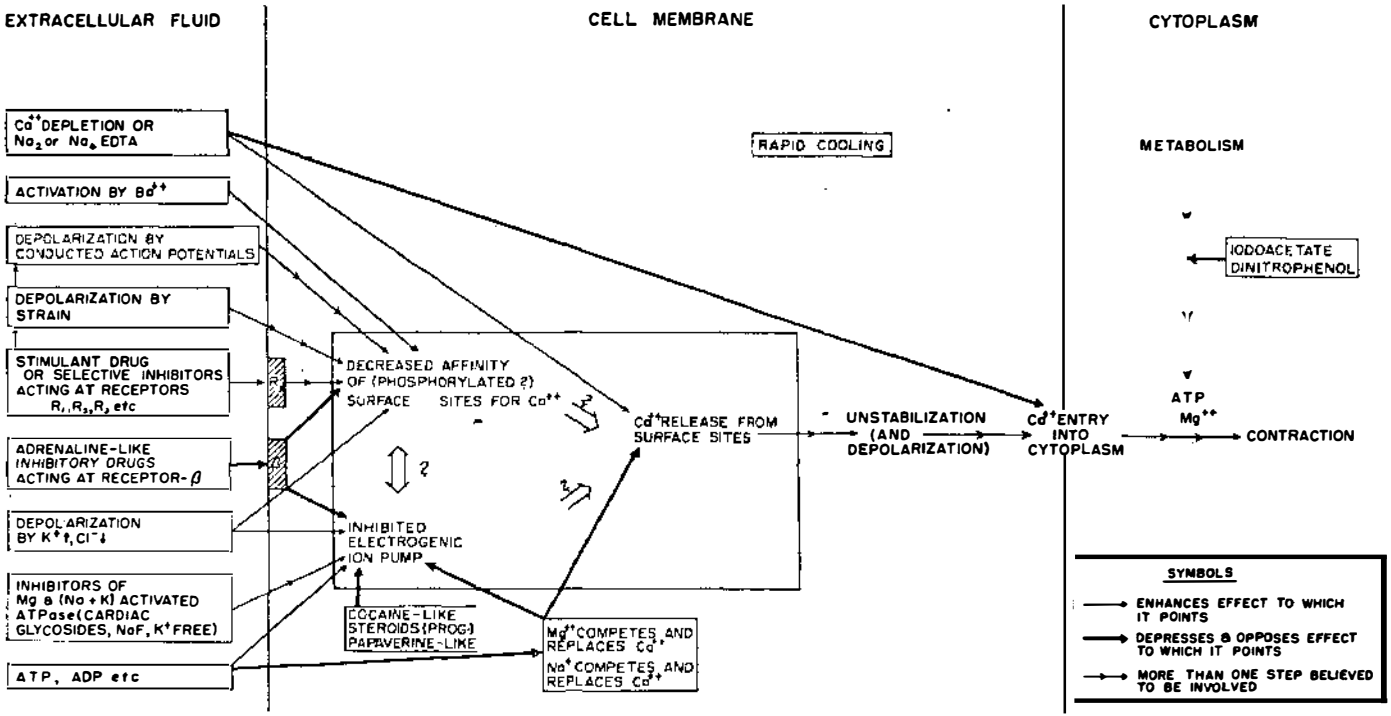

FIG. 1

Magnesium and, under some circumstances, sodium appear to compete with calcium opposing its contractile effect during chemical stimulation. Magnesium may replace Ca^{++} at some superficial binding sites and prevent Ca^{++} release and contractions because of a more stable complexing to these sites. Barium ions, in the presence or absence of Ca^{++} initiate contractures and appear to form an easily dissociable complex at surface sites where calcium combination leads to membrane stabilization. If combination at these sites is equivalent to stabilization it is difficult to explain how barium displaces calcium and stimulates contraction when both are present. Barium, as suggested in the diagram, may act indirectly by influencing affinity for Ca^{++}. On the other hand, Ba^{++}, though dissociating readily from the sites which oridinarily complex calcium, may also associate readily and successfully displace calcium, If such is the case, the arrow from Ba^{++} should have been drawn to the point labelled "Ca^{++} release, etc."

Rapid cooling acts at some site distal to cocaine since the contracture it produces is not prevented by cocaine. Its actions on the pump mechanism are not known to be related to its contractile effects.

Calcium entry into the cytoplasm triggers contraction when the requisite conditions (as to ATP and Mg etc.) are present. Inhibitors such as iodoacetate and dinitrophenol prevent contraction by cutting off the supply of ATP.

One important problem has been omitted from the scheme for lack of a simple hypotheseis which includes it. The interrelation between epinephrine inhibitory effects and carbohydrate metabolism is believed to involve a glucose transport mechanism at the cell membrane, but attempts to carry the proposal further at the present stage of knowledge soon became entangled in complex alternate hypothesis.

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