

*Anesthetic Depression of Myocardial Contractility:
A Review of Possible Mechanisms*

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CONTENTS

Electromechanical Coupling in Cardiac Muscle
Contractile Proteins
Activation of the Actomyosin Interaction by Calcium
Modulation of Calcium Activation of Actomyosin
Control of the Myoplasmic Calcium Concentration
Sarcolemma
Sarcoplasmic Reticulum
Mitochondria
Models for Control of Myoplasmic Calcium
Basis for Changes in Myocardial Contractility
Species and Tissue Variation
Variation in the Rate and Pattern of Stimulation
Change in Myocardial Fiber Length
Acidosis
Extracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_o$)
Catecholamines
Methylxanthines
Bipyridines
Digitalis
Anesthetic Depression of Cardiac Contractility
Mitochondria
Contractile Proteins
Sarcolemma: Electrical Activity
Sarcolemma: Ion Exchange
Sarcoplasmic Reticulum
Isolated Intact Myocardium
Summary

KNOWLEDGE OF THE contractile activation process in heart muscle has increased significantly during the past

2 decades,¹⁻³ allowing experimental studies to provide an increased understanding of the mechanisms underlying the well-appreciated negative inotropic effect of general anesthetics. In this review, we focus on the direct negative inotropic effect of these agents. We have excluded from discussion indirect effects on the myocardium, such as those mediated by the nervous system or those which are secondary to changes in cardiac preload or afterload.⁴ We begin with a review of the process of contractile activation.

Electromechanical Coupling in Cardiac Muscle

Electromechanical coupling has been defined as the series of events which begins with depolarization of the muscle cell membrane and ends with the production of force and movement.¹ Figure 1 illustrates the anatomical relationship between the cellular organelles which are involved in this process. A description of the events which occur in electromechanical coupling will facilitate further discussion of the sites at which anesthetics may act to alter the myocardial force of contraction.

CONTRACTILE PROTEINS

The generation of contractile force ultimately depends upon a cyclic interaction between actin and myosin molecules which are organized in a lattice of thick (myosin) and thin (actin) filaments in the myocardial cell. This interaction takes place between the myosin heads or crossbridges and the individual actin molecules of the myofilaments (fig. 2). The interaction is not well understood, but it is known to involve the splitting of MgATP^{-2} by an ATPase located in the head regions of the thick filaments. Transduction of the free energy thus derived results in movement and/or force production secondary to a sliding of the thick and thin filaments relative to one another.^{5,6} It is presently believed that this sliding motion is produced by a rotation of the myosin heads as they pivot on attachments they have made to the actin monomers of the thin filaments. Binding of ATP to the myosin head is associated with a loose binding (allowing detachment and re-attachment) of the

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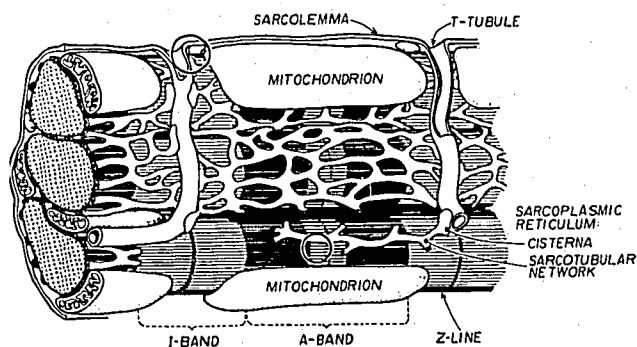


FIG. 1. Ultrastructure of a myocardial contractile cell. Contractile proteins (thick and thin filaments), sarcolemma and invaginating t-tubules, sarcoplasmic reticulum and mitochondria are depicted. The thick and thin filaments are arranged in hexagonal arrays in a longitudinal repeating fashion, forming the myofibrils. Z-lines demarcate the boundaries of each sarcomere (the basic contractile unit). The A-band is that portion of the sarcomere in which the thick and thin filaments overlap. The sarcolemma (cell membrane) provides a barrier which controls ionic fluxes between the inside and outside of the cell. Inward sarcolemmal invaginations, the t-tubules, extend sarcolemmal function into the interior regions of the cell. The sarcoplasmic reticulum (SR) is an internal system of membranes which provides intracellular Ca^{2+} -uptake and Ca^{2+} -release functions. Portions of this membrane are in close approximation with either the t-tubule (terminal cisternae) or the sarcolemma (sub-sarcolemmal cisternae). Reprinted from Katz AM: Congestive heart failure: Role of altered myocardial cellular control. N Eng J Med 293:1184, 1975, with permission of the author and The New England Journal of Medicine.

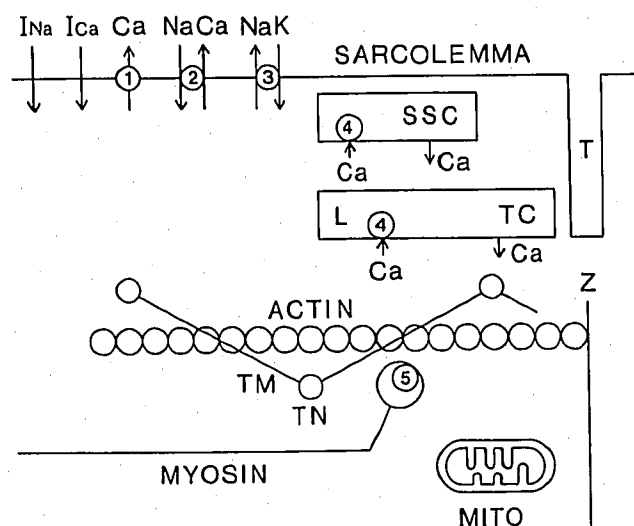


FIG. 2. Schematic diagram of actin and myosin filaments, regulatory proteins, and the organelles which control myocardial ion fluxes. Z = Z line of sarcomere; TN = troponin; TM = tropomyosin; T = transverse tubule; TC = terminal cisternal portion of sarcoplasmic reticulum (SR); L = longitudinal portion of SR; SSC = sub-sarcolemmal cisternal portion of SR; 1 = sarcolemmal Ca^{2+} -ATPase; 2 = sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchange mechanism; 3 = sarcolemmal Na^+/K^+ pump; 4 = SR Ca^{2+} -ATPase; 5 = myosin ATPase; I_{Na} and I_{Ca} = inward sodium and calcium currents.

crossbridge to actin and the hydrolysis of ATP to ADP + inorganic phosphate. Release of the ATP hydrolysis products results in a strong binding of the crossbridge to actin which is accompanied by rotation of the myosin head, i.e., the "power stroke." Binding of another ATP to myosin initiates the next cycle of myosin crossbridge-actin interaction and its accompanying power stroke.⁵ In the relaxed state (in the absence of Ca^{2+} , *vide infra*), a regulatory protein, tropomyosin, occupies a position on the thin filament such that it "covers" the binding sites of the actin monomers and thus prevents the binding of myosin to actin and the cycle just described from occurring.⁵⁻⁷ A second regulatory protein, troponin, is capable of altering the conformational state of tropomyosin such that the actin binding sites are exposed.^{5,6} Troponin consists of three subunits, an inhibitory subunit (TNI), a subunit directly associated with tropomyosin (TNT), and a Ca^{2+} -binding subunit (TNC).⁶

ACTIVATION OF THE ACTIN-MYOSIN INTERACTION BY Ca^{2+}

When the Ca^{2+} -binding site on TNC is occupied, tropomyosin ceases to interfere with the binding of myosin to actin, allowing contraction to occur.^{6,7} The degree of activation of contraction depends upon the concentration of ionized calcium in the myoplasm ($[\text{Ca}^{2+}]_i$).⁸ Below a Ca^{2+} concentration of 10^{-7} M, there is no activation (diastole), while, at a concentration of approximately 10^{-5} M, activation is maximum.⁹ Over this Ca^{2+} concentration range, both ATP hydrolysis and contractile force vary, sigmoidally, from essentially zero to maximum (fig. 3).¹⁰ This sharp (sigmoidal) rise in force over a narrow range of increased Ca^{2+} concentration suggests that either cooperativity or multiple Ca^{2+} binding sites on troponin is involved.¹ Of these, cooperativity is most likely, since there is only one regulatory site (functional Ca^{2+} receptor) on cardiac troponin.⁶ In heart muscle, the systolic concentration of myoplasmic Ca^{2+} is, normally, well below that needed for full activation.⁸ Variation of the myoplasmic concentration of this ion is thought to play a very important role in the control of myocardial contractile force.⁹⁻¹¹

MODULATION OF Ca^{2+} ACTIVATION OF ACTOMYOSIN

While the Ca^{2+} concentration in the myoplasm is of major importance for the control of myocardial contractility, evidence is accumulating that the contractile proteins can themselves undergo changes which result in the modulation of contractile force. These changes occur by virtue of phosphorylation reactions involving cyclic AMP (cAMP)-dependent protein kinases, and have been demonstrated in the laboratory to result in

the alteration of both maximum force development and the sensitivity of the contractile proteins to activation by calcium.¹²

Phosphorylation of the inhibitory subunit of troponin (TNI) has been shown to result in displacement of the curve of force *versus* $[Ca^{2+}]_i$ to the right, *i.e.*, to decreased Ca^{2+} sensitivity, without an alteration of maximum Ca^{2+} activated force.¹³ The decrease in Ca^{2+} sensitivity is caused by a faster rate of release of Ca^{2+} by troponin,¹⁴ which, in turn, is thought to be partially responsible for the increased rate of relaxation observed when TNI is phosphorylated.¹³ Dephosphorylation of TNI is thought to be brought about by a cGMP-dependent reaction which may be controlled by the cholinergic system.¹⁵

Cyclic-AMP-dependent phosphorylation of an, as yet, unidentified intracellular protein has been shown to produce an active subunit which alters myosin such that an increase in Ca^{2+} activated maximum force development, and actin-activated ATP-ase activity, results.¹⁶ Several isozymes of myosin are known to exist,¹⁷ and the one named VI is subject to alteration in this manner.¹² There is experimental evidence which suggests strongly that activation of this VI isozyme is involved in the positive inotropic response to drugs which increase cellular levels of cAMP.¹⁸

CONTROL OF THE MYOPLASMIC Ca^{2+} CONCENTRATION

It is generally agreed that the calcium ion is the most important mediator of acute changes in myocardial contractile activation.¹ Evidence obtained from "skinned" cell preparations,^{9,10,19,20} and from studies employing Ca^{2+} -sensitive microelectrodes,²¹ indicates that the myoplasmic Ca^{2+} concentration lies in the micromolar or submicromolar range. Even at the height of activation, the myoplasmic Ca^{2+} concentration is several orders of magnitude less than the concentration of Ca^{2+} which exists extracellularly, so that mechanisms must exist to maintain that gradient while, at the same time, allowing for cyclic alterations in the myoplasmic Ca^{2+} concentration which occur between rest and full activation. These mechanisms mainly involve membrane-bound calcium translocating proteins (pumps). Of these, the ones residing in the sarcolemma and sarcoplasmic reticulum (SR) appear to bear the major responsibility for beat-to-beat control of calcium, while those located in mitochondria may be important for long-term adjustments of the intracellular Ca^{2+} milieu or for cellular protection during periods of Ca^{2+} overload.^{1,22} Soluble proteins, such as calmodulin, which are capable of binding Ca^{2+} in the micromolar concentration range also serve to buffer changes in intracellular

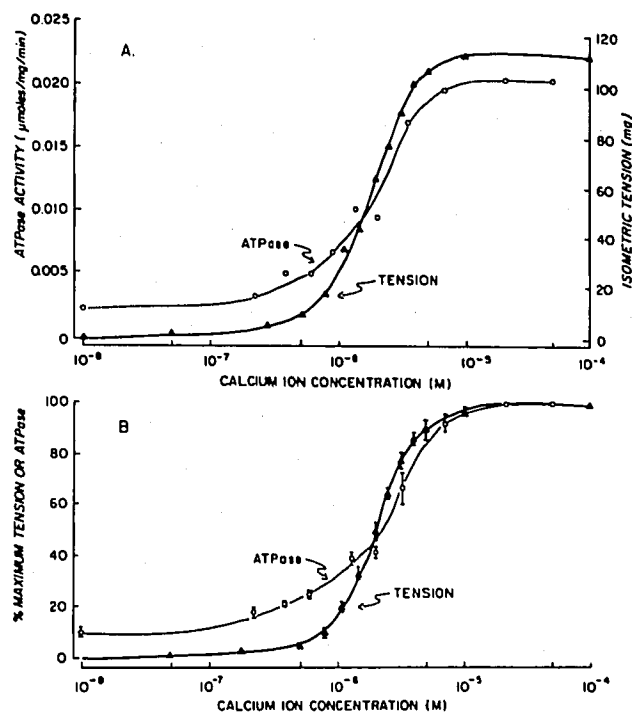


FIG. 3. Sigmoidal relationship between myoplasmic calcium ion concentration (M), and isometric developed tension and actomyosin ATPase. A. Typical experiment. B. Normalized tension and ATPase activity for several papillary muscles. Note that major change in both tension and ATPase activity takes place when myoplasmic Ca^{2+} concentration increases from approximately $10^{-6.5}$ to $10^{-5.5}$. Reprinted from Solaro RJ *et al*: Calcium requirements for cardiac myofibrillar activation. *Circ Res* 34:525-530, 1974, with permission from the author and The American Heart Association, Inc.

Ca^{2+} , although their major purpose is to modulate functions which either control, or are controlled by, Ca^{2+} .²³ Calmodulin's function in heart muscle has not been well defined, except for a possible role in the phosphorylation of the SR (*vide infra*).

Sarcolemma. The sarcolemma controls both the influx and the efflux of Ca^{2+} (fig. 2). The transsarcolemmal influx of Ca^{2+} occurs passively down the Ca^{2+} concentration gradient noted above through channels which open during sarcolemmal depolarization. The transmembrane resting potential (fig. 4) is approximately -90 millivolts in ventricular contractile cells. Depolarization (phase 0 of the action potential) is initially accomplished *via* the influx of Na^{+} through specific "fast" sodium channels. As the membrane potential approaches -30 millivolts, the Ca^{2+} channels begin to open and are open maximally at a potential of +30 millivolts.²⁴ The influx of calcium ions, the so-called slow inward current (I_{Ca} in fig. 2, classically labeled I_{si}), contributes to the depolarization of the cell membrane, ultimately accounting in part for a portion of the plateau (phase 2) of the action potential. Experiments em-

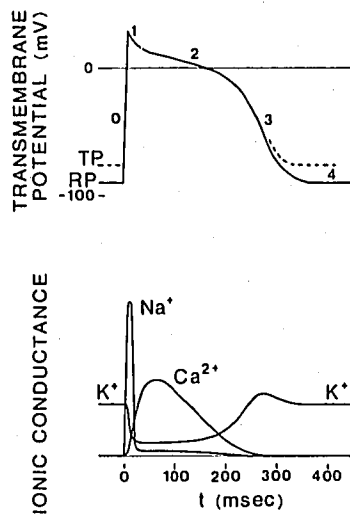


FIG. 4. Schematic representation of the transmembrane potential of a non-automatic ventricular contractile cell with accompanying changes in sarcolemmal ion conductances. Phases of the action potential are listed (0 through 4). During rest (phase 4), K⁺ conductance predominates and K⁺ efflux is primarily responsible for the resting potential (RP). When the membrane potential is brought to threshold (TP), Na⁺ conductance rapidly increases and the resulting influx of Na⁺ produces the rapid depolarization phase (phase 0) of the

action potential. Na⁺ influx begins to decline when the transmembrane potential becomes more positive than -50 mV, at which point Ca²⁺ conductance increases and Ca²⁺ influx (the so-called Ca²⁺ slow inward current) begins to contribute to membrane depolarization. The plateau (phase 2) of the action potential is determined by a combination of Ca²⁺ influx, declining Na⁺ influx, and (anomalous) K⁺ efflux. K⁺ efflux and declining Ca²⁺ influx is responsible for the rapid final repolarization phase (phase 3). While K⁺ conductance is depicted as a single entity, there are, in reality, several K⁺ currents. The initial rapid repolarization phase (phase 1) is variably present in ventricular cells, and is due to a combination of several changing ionic conductances.

playing microelectrodes in which a small portion of sarcolemma is voltage clamped (patch clamp technique) have shown that approximately 10^3 Ca²⁺ ions enter through each channel during an opening event, and that the mean open time of a channel is approximately 1 msec.²⁴ Cyclic-AMP-dependent phosphorylation of Ca²⁺ channel proteins (which can be induced, for example, by catecholamines) increases the probability of channel openings and the number of calcium ions entering the cell during depolarization.²⁵ Controversy exists²⁶⁻²⁸ concerning whether the amount of calcium which enters the cell during depolarization is sufficient to effect full contractile activation, or whether supplementation from another source, *e.g.*, sarcoplasmic reticulum, is necessary.^{1,2,11}

In the steady state, the amount of Ca²⁺ entering the cell must be matched by the amount extruded if Ca²⁺ overload is to be avoided.^{22,29} Two systems exist in the sarcolemma for the outward transport of Ca²⁺. One is a high affinity ATP-ase which can extrude Ca²⁺ from the myoplasm, even when its concentration is very low, as in diastole.³⁰ The other, a lower affinity Na⁺/Ca²⁺ exchange system,^{22,29,31} exchanges three Na⁺ for each Ca²⁺, deriving energy for Ca²⁺ efflux from the transmembrane gradient for Na⁺. Na⁺/Ca²⁺ exchange is

electrogenic (*i.e.*, three univalent Na⁺ exchanged for a single divalent Ca²⁺), so that Ca²⁺ may actually be transported inwardly at the height of depolarization when the transmembrane potential is inside positive (fig. 3) and favors this direction of exchange.^{31,32} It should, however, be mentioned that arguments have been advanced against Na⁺/Ca²⁺ exchange ever accounting for inward Ca²⁺ transport.³³

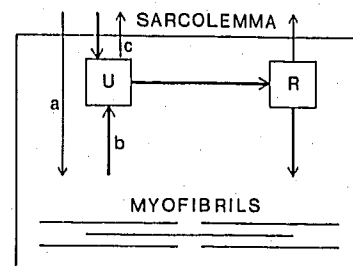
Sarcoplasmic Reticulum. The sarcoplasmic reticulum (SR) is an internal system of tubular membranes consisting of two components, the free (longitudinal) and the junctional (terminal cisternal) SR.³⁴ It functions both to sequester and to release intracellular Ca²⁺. Ca²⁺ uptake (translocation) occurs against a concentration gradient of approximately 10^3 , and is accomplished by an active transport system (pump) consisting of a Ca²⁺ specific ATP-ase.^{1,2} Two Ca²⁺ are sequestered for each molecule of ATP hydrolyzed.³⁵ A protein, calsequestrin, located in the SR lumen, acts to bind translocated Ca²⁺, thereby reducing the gradient against which the pump works.³⁶ Both the rate and capacity of this system are sufficient to account for beat-to-beat regulation of contractility.^{1,2} Phosphorylation of a third protein, phospholamban, mediated *via* cAMP-dependent and Ca²⁺-calmodulin-dependent protein kinases, results in increased activity of the system.^{2,37} In mammalian cardiac muscle, the junctional SR forms couplings with both the surface plasmalemma and with inward extensions of the plasmalemma, the transverse tubules (fig. 1). These couplings have been postulated to provide a means whereby the action potential of the plasmalemma can induce junctional SR Ca²⁺ release. However, much of the junctional SR is devoid of these couplings,³⁴ and controversy exists as to the exact mechanism responsible for the triggering of SR Ca²⁺ release. Proposed mechanisms include the electrical transmission of the plasmalemmal signal to the SR (presently thought unlikely³⁸), and transmission *via* the calcium ion itself, the so-called Ca²⁺-induced release of SR Ca²⁺.^{39,40} Most recently, transmission by a second messenger, inositol triphosphate (IP₃), has been proposed.^{38,41} It has been demonstrated that stimulation of various receptors, including adrenergic, muscarinic, and histaminic, is associated with an increase in membrane phospholipase C which results in the formation of IP₃ from phosphatidylinositol. IP₃ has been shown to enhance Ca²⁺ release from the SR of both skeletal and cardiac muscle.⁴¹ In any event, the amount of Ca²⁺ released to the myoplasm by the SR appears to depend on the amount of Ca²⁺ stored in this organelle, which, in turn, is a function of factors such as the extracellular Ca²⁺ concentration,⁴² the frequency and character of stimulation of the cell,⁴³ the degree of phosphorylation of the SR membrane,^{2,22} and the amount of Ca²⁺ lost from sequestration during

rest.⁴⁴ The SR is capable of releasing sufficient Ca^{2+} for full contractile activation.^{1,9}

Mitochondria. Mitochondria sequester Ca^{2+} by a low affinity electrophoretic process which is only weakly activated by the sarcoplasmic Ca^{2+} levels normally present in heart.²² The submicromolar intracellular Ca^{2+} concentrations seen in diastole are not dependent on mitochondrial uptake.⁴² As mentioned earlier, the mitochondria apparently do not participate in beat-to-beat cytosolic Ca^{2+} regulation under normal circumstances,⁴⁰ and only become important in pathologic situations which feature the intracellular accumulation of abnormally large amounts of Ca^{2+} .⁴⁵

Models for Control of Myoplasmic Ca^{2+} . While it is generally agreed that the Ca^{2+} for activation of contraction is supplied *via* transsarcolemmal influx and internal SR release,¹¹ the extent to which each of these Ca^{2+} sources participates in a given contractile event is still a matter of controversy. Debate centers around whether the amount of transsarcolemmally derived Ca^{2+} is sufficient for full activation of the myofibrils, whether transsarcolemmally derived Ca^{2+} directly activates actomyosin,²⁸ whether it must pass through the SR before doing so,⁴⁶ or whether it acts primarily to trigger the internal release of Ca^{2+} from the SR.^{28,40} Also, it is not known whether the SR consists of separate compartments for Ca^{2+} uptake and Ca^{2+} release with transport between compartments, or whether a single compartment serves both of these functions.^{43,47} Furthermore, Bers,²⁸ Sutko and Willerson,⁴⁸ and Fabiato,⁴⁰ among others, have suggested that the mechanism of contractile activation varies with the species, the tissue (*i.e.*, atrial or ventricular), and the type of stimulation. Figure 5 illustrates a model for electromechanical coupling proposed by Edman and Jóhannsson.⁴³ It is shown only as an example, and can, with modification, encompass other proposed models. In this model, the SR is composed of both uptake and release compartments, and it is suggested by Edman and Jóhannsson⁴³ and others¹¹ that these are represented, respectively, by the longitudinal SR and by the portions of the SR designated as lateral sacs and subsarcolemmal cisternae. Ca^{2+} which is sequestered at the SR uptake site is transported within the SR, in a time-dependent manner, to the release compartment from which it can then be released to activate the myofibrils during a subsequent action potential. This model is kinetically indistinguishable from one in which the SR is comprised of a single compartment, wherein the time required before release can be accomplished is determined by a time-dependent process of recovery of activation of Ca^{2+} release channels,⁴⁷ rather than by a time-dependent process of intra-SR transport. While Edman and Jóhannsson suggest that the Ca^{2+} which enters the cell during the action potential is, in

FIG. 5. A model for myocardial Ca^{2+} fluxes. After scheme proposed by Edman and Jóhannsson,⁴³ U = Ca^{2+} uptake site, and R = release site of the sarcoplasmic reticulum, based on Edman and Jóhannsson's work with potentiated-state contractions. Pathways for major Ca^{2+} fluxes indicated by bold arrows. Another model, proposed by Bers,²⁸ can be visualized by assigning, instead, a, b, and c as the major Ca^{2+} fluxes. Note, also, that the Ca^{2+} uptake and release sites have been located in a single compartment in another proposed model.⁴⁷



large part, sequestered at the SR uptake site with only a small amount reaching the myofibrils for direct activation, transsarcolemmally derived Ca^{2+} may, as stated above, all traverse the SR before reaching the myofibrils,⁴⁶ or may, in large part, reach the myofibrils directly.^{29,47}

Basis for Changes in Myocardial Contractility

Cardiac contractile force is capable of wide variations in intensity. As indicated above, changes in contractile force may be brought about by changes in the cytosolic activator Ca^{2+} concentration and/or by changes in the contractile proteins which alter their responsiveness to activator Ca^{2+} .

SPECIES AND TISSUE VARIATION

The degree to which externally derived or internally released Ca^{2+} participates in contractile activation varies greatly with the species and with the tissue (*i.e.*, atrium *vs.* ventricle). In frog ventricle, contractile activation depends almost entirely on Ca^{2+} -derived transsarcolemmally, while, in rat, the important calcium source is the SR. Rabbit, guinea pig, cat, and dog lie between these extremes in the order listed.⁴⁸⁻⁵⁰ Human ventricle appears to lie between rabbit and cat, at least in regard to the threshold for Ca^{2+} -induced release of Ca^{2+} from the SR.⁵¹ Atrium appears, generally, to depend mostly upon internal sources of Ca^{2+} for activation. The efficiency of calcium-induced calcium release in skinned cardiac muscles varies depending on the species, decreasing in the order of rat, dog, cat, and rabbit.⁵¹ Also, Nayler *et al.*⁵² have demonstrated a higher rate of uptake and release of Ca^{2+} by SR from rat as compared to guinea pig and rabbit. Such variations may underlie the functional differences seen between these species. Extrapolation of observed drug effects from one species to another must take these differences into account.

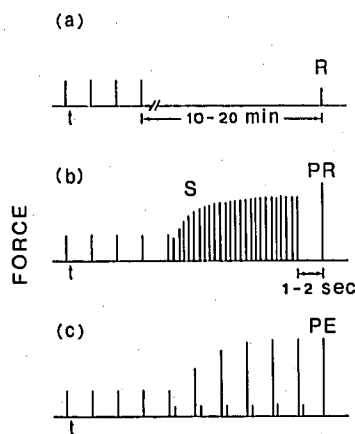


FIG. 6. Effect of rate and pattern of stimulation on contractile state. (a); Rested-state contraction (R) is elicited by a stimulus which follows a long (10-20-min) rest period. During the rest, internal store of SR Ca^{2+} is depleted. Rested-state contraction is activated predominantly by Ca^{2+} supplied via transsarcolemmal influx. (b); Staircase (S) is also called positive staircase, Bowditch staircase, and Treppe. Force of contraction increases to a

new steady-state over several beats following an abrupt transition to a higher rate of stimulation. Post-rest (PR) potentiated-state contraction is elicited by a stimulus delivered after 1-2 s of rest following a train of high frequency stimuli. (c); Postextrasystolic potentiation (PE) is elicited in response to paired-pulse stimulation. The intrapair stimulus interval is approximately 300 msec. Ca^{2+} released internally from the SR assumes a major role in the activation of potentiated-state contractions.

VARIATION IN THE RATE AND PATTERN OF STIMULATION

Contractile force is influenced by the rate and pattern of stimulation, as is the degree to which transsarcolemmally derived or internally released Ca^{2+} participates in contractile activation (fig. 6). During prolonged rest of isolated ventricular muscle from most mammalian species, the SR is essentially depleted of its intracellular store of Ca^{2+} . A subsequent action potential results in a weak, "rested state" contraction, which is triggered by direct action of the small portion of transsarcolemmally derived Ca^{2+} that enters the cell and is not sequestered by the SR.^{43,44} Note that Reiter *et al.*⁴⁶ propose that this transsarcolemmally derived Ca^{2+} passes through the SR before activation occurs. That rested state contractions are dependent upon the transsarcolemmal influx of Ca^{2+} for their activation is evidenced by the fact that they are strongly suppressed by Ni^{2+} and by other inhibitors of the slow inward Ca^{2+} current.^{44,46,53} Opposite to the rested state is the forceful potentiated state contraction, which can be produced by paired-pulse stimulation (post-extrasystolic potentiation),⁴⁴ or by stimulation after brief rest following high frequency stimulation (post-rest potentiation).⁴³ The time required for maximal potentiation to develop apparently depends upon the time needed for transport of Ca^{2+} within the SR,⁴³ or, alternatively, for the recovery of activation of SR Ca^{2+} release channels.⁴⁷ These potentiated state contractions depend mainly upon Ca^{2+} derived internally from SR release for their activation, as is indicated by

the observation that they are strongly inhibited by ryanodine,^{28,54} a drug which specifically inhibits SR function.⁵⁵ Subservient by possibly a still different mechanism is the increase in contractile force which accompanies an increase in the frequency of beating (the positive staircase). The Ca^{2+} supporting this rate-related inotropic event may come from the SR, but the mechanism underlying Ca^{2+} storage and release may be different from that involved in potentiated state, as: 1) staircase is less sensitive to inhibition by ryanodine than is potentiated state,⁵⁴ 2) staircase can be produced in amphibian ventricle which is almost devoid of SR,⁴⁹ and 3) the Ca^{2+} in superficial sarcolemmal sites increases when the beat frequency is increased.⁵⁶ Also of interest in this regard is the observation that rat ventricle, which exhibits potentiation, develops *decreased* force with increased frequency of stimulation, i.e., it manifests a *negative* staircase.⁴⁸ It should be noted that, at this time, the source of activator Ca^{2+} for staircase is in dispute, and that there may be a number of contributing sources. Changes in stimulus frequency or in the pattern of stimulation, e.g., paired-pulse stimulation, do not alter the sensitivity of the myofilaments to Ca^{2+} .⁵⁷

CHANGE IN MYOCARDIAL FIBER LENGTH

The increase in developed force which accompanies an increase in diastolic fiber length, the length-tension or Frank-Starling relation, is well known. The cellular basis for this relationship was originally thought to reside in an alteration of myofilament overlap, but it is now believed that this mechanism plays only a small role, while length-related changes in activation are of major importance. These changes in activation include increases in the amount of internally released Ca^{2+} and in the sensitivity of the myofibrils to Ca^{2+} with increasing muscle length. Of these, the change in myofilament sensitivity is thought to be of greater importance, although accurate quantitation of the individual contribution of each process in the intact cell is not presently possible.⁵⁸ The change in myofilament Ca^{2+} sensitivity is apparently not due to TNI dephosphorylation, since the phenomenon occurs in situations in which the relevant phosphorylation mechanisms have been deactivated.¹²

ACIDOSIS

A change in intracellular pH from 7.0 to 6.2 causes a decrease in the sensitivity of the myofilaments to Ca^{2+} . It also decreases the maximum tension developed at saturating levels of $[\text{Ca}^{2+}]$. Acidosis decreases the ability of the SR to sequester and release Ca^{2+} .⁵⁹

EXTRACELLULAR Ca^{2+} CONCENTRATION ($[\text{Ca}^{2+}]_o$)

The importance of extracellular Ca^{2+} for cardiac function has been appreciated since Sidney Ringer's time.⁶⁰ Removal of external Ca^{2+} causes cardiac contractile force to decline exponentially with a $t_{1/2}$ of approximately 50 s, while replenishment of Ca^{2+} results in the return of force with a time course which is three to five times faster.⁶¹ Changing $[\text{Ca}^{2+}]_o$ does not alter the relationship between $[\text{Ca}^{2+}]_i$ and contractile force, *i.e.*, this maneuver apparently does not change the sensitivity of the myofilaments to Ca^{2+} .⁵⁷

CATECHOLAMINES

The positive inotropic effect of catecholamines is the result of several subcellular actions, all of which are mediated *via* stimulation of the adenylate cyclase system and subsequent increases in cAMP-dependent protein kinase activity.⁶² This activity causes phosphorylation of a variety of proteins, including those in sarcolemma, SR, and the myofibrils. At the sarcolemma, catecholamines increase the influx of Ca^{2+} , as demonstrated by increase of the slow inward Ca^{2+} current in voltage clamp studies of multicellular and patch-clamped preparations.^{24,25,29,63} Likewise, these agents also increase the plateau phase of the action potential^{64,65} and the force of the slowly rising rested state contraction,⁶⁶ providing further evidence that they increase Ca^{2+} influx. It has been postulated that these effects occur secondarily to phosphorylation of Ca^{2+} channel membrane proteins.^{25,67} Catecholamine-induced phosphorylation of the SR results in increased rate and capacity of sequestration of Ca^{2+} by this organelle.^{37,68} As a consequence, more Ca^{2+} is available for internal release providing increased force of contraction, while, at the same time, relaxation is hastened.^{39,57} As mentioned earlier, phosphorylation of TNI mediated by cAMP-dependent protein kinase results in decreased Ca^{2+} sensitivity of the myofibrils. This has been demonstrated in the intact cell using aequorin to monitor changes in $[\text{Ca}^{2+}]_i$.⁵⁸ The catecholamine-induced decrease in Ca^{2+} sensitivity is, however, more than compensated for by the concomitant increase in myoplasmic $[\text{Ca}^{2+}]$.¹²

METHYLYXANTHINES

Caffeine and theophylline exert numerous actions in the myocardial cell, some of which enhance, and some which depress, contractility.⁶⁹ Ca^{2+} influx during the plateau of the action potential is increased,²⁹ most likely *via* a cAMP-mediated action which occurs secondary to the inhibition of phosphodiesterase.⁶⁸ The sensitivity of the myofilaments to Ca^{2+} is slightly enhanced,^{70,71} causing the $[\text{Ca}^{2+}]_i$ -tension curve to be shifted to the left.⁵⁷

Another major action of these drugs is the inhibition of Ca^{2+} sequestration by the SR which can result in a retardation of relaxation. This action is, more than likely, not mediated *via* cAMP.⁶⁸ Contractility may be enhanced^{65,69} or depressed⁷¹ by the methylxanthines.

BIPYRIDINES

The bipyridine, amrinone, has recently been introduced for the treatment of heart failure. It apparently acts through phosphodiesterase inhibition to cause increase in cellular cAMP with subsequent increase in contractility.^{72,73}

DIGITALIS

The cardiac glycosides act exclusively by increasing $[\text{Ca}^{2+}]_i$. They apparently do not alter the Ca^{2+} kinetics of the SR or the Ca^{2+} sensitivity of the myofilaments.⁷⁴ Inhibition of the Na/K-pump⁷⁵ has been suggested as a mechanism responsible for the increase in $[\text{Ca}^{2+}]_i$. This inhibition causes a net gain of Na^+ and, secondarily, an increase in $[\text{Ca}^{2+}]_i$ *via* $\text{Na}^+/\text{Ca}^{2+}$ exchange.²⁹ While this mechanism may be operative at high ($>10^{-8}$ M) concentrations of cardiac glycosides, lower, *therapeutic* concentrations of cardiac glycosides show positive inotropic effects without inhibition of Na/K-ATPase, suggesting that another mechanism, such as the alteration of sarcolemmal membrane structure to increase the amount of bound Ca^{2+} , may be important.⁷⁴ Digitalis does not alter the $[\text{Ca}^{2+}]_i$ -tension relationship.⁵⁷

Anesthetic Depression of Cardiac Contractility

From the foregoing discussion, it is clear that there are a number of subcellular sites at which anesthetics might act to suppress the heart's ability to contract. These include the contractile proteins, the mitochondria, the sarcoplasmic reticulum, and the sarcolemma. All of these have been examined, albeit to varying extent, for their possible role in the phenomenon of anesthetic negative inotropism.

MITOCHONDRIA

Berman *et al.*⁷⁶ suggested that a decrease in the synthesis of ATP consequent to halothane's inhibitory effect on the mitochondrial oxidation of NADH-linked substrates could explain the anesthetic's depressant effect on myocardial contractility. Although halothane,⁷⁷⁻⁷⁹ enflurane, methoxyflurane, diethyl ether,⁸⁰ isoflurane, and fluroxene⁸¹ do, in isolated mitochondria, cause a dose-related depression of state 3 (ADP-dependent) NADH-linked glutamate oxidation, which is reversible for clinically employed doses of these anesthetics, the oxidation of succinate (a substrate which

bypasses the NAD/NADH step in the mitochondrial electron transport chain) is little affected. Based on these observations, Merin⁸² suggests that it is unlikely that decreased ATP synthesis is responsible for depression of contractile function. This is further supported by his observation that myocardial tissue levels of ATP, as well as creatine phosphate and glycogen, are maintained normal during halothane anesthesia in the pig. In the isolated intact heart, anesthetic depression of glutamate oxidation measured by means of NADH fluorometry is minimal.⁸³ NADH fluorometry, a technique devised by Chance *et al.*⁸⁴ to assess the oxidation-reduction state of intact tissues, is based on the fluorescence of NADH at 465 nm, which is absent in the oxidized (NAD) state. Such fluorescence is maximal under the condition of tissue anoxia. Kissin *et al.*⁸³ found that the anesthetic concentrations needed to cause 10% of the increase in NADH fluorescence caused by anoxia were 1.1% halothane, 4.6% isoflurane, 2.6% enflurane, and 6.8% ether. These authors also observed that, for the anesthetics tested, the increase in NADH fluorescence did not correlate well with depression of contractile function. This, they felt, supported the idea that depression of myocardial contractility by volatile anesthetics is not caused by inhibition of energy production. Although, at this time, it is not possible to state definitely that depression of energy production by anesthetics does *not* contribute to their observed negative inotropic effect, such a relationship must be considered highly unlikely. The fact that both halothane⁸⁵ and enflurane⁸⁶ markedly inhibit contractile force in functionally skinned myocardial fibers where ATP levels are experimentally maintained suggests that decreased ATP synthesis is at least not a *necessary* condition for anesthetic negative inotropism.

Anesthetic actions in mitochondria which alter their Ca^{2+} homeostasis could, conceivably, also affect myocardial contractile function. Miller and Hunter⁷⁸ have shown that halothane slows the rate of mitochondrial Ca^{2+} accumulation, but not the final amount accumulated. If the myoplasmic $[\text{Ca}^{2+}]$ was increased due to inability of the mitochondria, in the presence of anesthetics, to sequester Ca^{2+} , contractile force and/or resting (diastolic) tension might be expected to increase. As is well known, myocardial developed force is generally decreased by anesthetics. While there is controversy regarding a direct effect of anesthetics on resting tension,^{87,88} it is possible that anesthetic-induced changes in diastolic compliance which occur in the intact ventricle may simply be secondary to changes in ventricular loading,⁸⁹ *i.e.*, to shifts of position on the non-linear diastolic length-tension curve. The submicromolar intracellular Ca^{2+} concentrations seen during diastole (Ca^{2+} levels affording relaxation) are not increased,

even if the Ca^{2+} uptake function of mitochondria is markedly impaired by the uncoupler FCCP.⁴² It is not likely that anesthetic effects on mitochondrial Ca^{2+} accumulation are a cause of negative inotropy.

CONTRACTILE PROTEINS

A number of investigators have examined myocardial actomyosin ATPase for its possible role in the phenomenon of anesthetic-induced negative inotropism. In an early study of this type, Brodtkin *et al.*⁹⁰ did demonstrate depression of ATPase activity by halothane in suspensions of rat cardiac myofibrils, but only at very high (20–100 mM) anesthetic concentrations. They observed a 45% decline in enzyme activity at a halothane concentration of 40 mM. It should be noted that a relevant gas phase clinical concentration of halothane, such as 1%, would, at equilibrium, produce a liquid phase concentration of approximately only 0.4 mM. Thus, a concentration of 40 mM is roughly two orders of magnitude higher than is clinically relevant. Another criticism of this work, pointed out by Merin,⁹¹ is that the Ca^{2+} concentration was not controlled (or known). Since Merin's subsequent work⁹² has shown that anesthetic effects on ATPase activity are Ca^{2+} dependent, the relevance of Brodtkin's study is difficult to assess. Shortly to follow was the study of Leuwenkroon-Stroberg *et al.*⁹³ of the effect of chloroform, diethyl ether, and halothane on myosin conformation and enzymatic activity. Halothane and chloroform (3 and 10%) and ether (10 and 30%) caused conformational changes in myosin, as indicated by changes in optical rotatory dispersion. The enzymatic changes observed were for Ca^{2+} activated myosin ATPase. Since the physiologically relevant reaction is the hydrolysis of MgATP^{-2} by actomyosin ATPase (actin-activated myosin ATPase), the enzymatic activity monitored by these workers probably has no physiological bearing. Indeed, they showed Ca^{2+} activated myosin ATPase to be stimulated by halothane and ether rather than depressed. Next to appear were the studies of Merin *et al.*⁹² and Ohnishi *et al.*⁹⁴ Merin's group observed a 36% depression of actomyosin ATPase activity in dog heart myofibrils by 4.4 mM halothane at a pCa^{2+} ($-\log_{10}[\text{Ca}^{2+}]$) of 6.5. The reader is again reminded that this concentration of halothane is approximately one order of magnitude higher than is clinically relevant. The halothane depression was completely reversed by Ca^{2+} concentrations of 10^{-3} to 10^{-2} M, and was abolished by rendering the actomyosin insensitive to Ca^{2+} (done by washing, heating, or aging the preparation, all of which removed or inactivated the troponin-tropomyosin regulator complex). Merin postulated that the reason that such high halothane concentrations were needed to show an effect was the fact

that the isolation process rendered his preparation relatively insensitive to Ca^{2+} and drugs. A similar insensitivity of this preparation has been noted for catecholamine analogs.⁹⁵ Ohnishi *et al.*⁹⁴ worked with cat heart myofibrils. They used sodium azide (as did Merin) to inhibit any contaminating mitochondrial ATPase. At a pCa^{2+} of 6.0, 1% halothane (gas phase) caused about a 20% inhibition of ATPase activity. Again, Ohnishi and Price found that removal of troponin-tropomyosin, by washing with NaHCO_3 , caused a loss of sensitivity to both Ca^{2+} and halothane. Reintroducing troponin-tropomyosin restored sensitivity. These authors postulated the site of the inhibitory action of halothane to be the troponin-tropomyosin complex, although Merin⁹¹ urged caution in naming a specific site, stating that studies of halothane's effect of troponin-tropomyosin Ca^{2+} binding would be necessary before this site of action for the anesthetic could be confirmed. A definitive study of the effect of anesthetics of TNC Ca^{2+} binding has yet to appear. The most recent report of the action of volatile anesthetics on myofibrillar ATPase is that of Pask *et al.*⁹⁶ Effects, at 5° C, of final concentrations of halothane (1.0 and 5.0 mM), isoflurane (0.8 and 4.1 mM), and enflurane (0.9 and 4.4 mM) were studied in a bovine preparation in which azide was present to inhibit contaminating mitochondrial ATPase activity. Anesthetics were added to the reaction mixture in liquid form, and the activating Ca^{2+} concentration was varied between 10^{-10} and 1.6×10^{-5} M. No depression of ATPase activity was observed with enflurane, while 5.0 mM halothane caused a 42% decrease in activity at a pCa^{2+} of 6.5. This result for halothane is in close agreement with Merin's⁹² observation. Again, it should be noted that, at 5° C, a 5-mM liquid phase concentration of halothane would equilibrate, roughly, to 5.5% in the gas phase. Depression by isoflurane was approximately one-half that observed with halothane.

The significance of the results of these investigations is difficult to determine. Certainly, the anesthetic concentrations which produced effects in these studies are high. In some instances, as already mentioned, the importance of the observations is diminished by absence of control of Ca^{2+} concentration, or work with a non-physiologically activated ATPase. In other instances, no experimental fault is obvious. In these cases, one is left with Merin's⁹² speculation that the anesthetic concentrations, though high, may be what actually exist in the microenvironment of the myofibrillar proteins (anesthetic partition coefficient unknown) of hearts perfused by media containing normal, clinically relevant concentrations of anesthetics. A caveat which may pertain here, however, is the fact that the specified concentrations of anesthetics are what were measured in the experimental media used. Since, for the most part, the

myofibril concentration in these media was in the range of 0.5–1.0 mg/ml, the anesthetic concentrations which existed in the myofibrillar proteins may have been even higher than those measured in the predominantly aqueous media. It should be stated here that it is the vapor pressure of a volatile anesthetic (given by its gas-phase concentration) which is the sole determinant of the anesthetic's thermodynamic activity in any phase of the experimental system. That is, if the vapor pressure is held constant, and if equilibrium with other phases is attained, then the anesthetic concentration in any given phase, *e.g.*, protein, is irrelevant.⁹⁷ Simply stated, it is of prime importance to know the *gas phase* concentration with which a system is in equilibrium. As mentioned above, Merin⁹² also suggests that the experimental extraction procedures employed render myofibrillar ATPase less sensitive to anesthetic depression. Support for this latter speculation comes from recent work of Winegrad *et al.*,¹² who have shown that maximum actin-activated myosin ATPase activity can be enhanced several fold when the VI isozyme of myosin predominates and is activated by a phosphorylated intracellular subunit. This form of regulation fails to survive isolation of actin and myosin from the cell. It is possible that such regulated actomyosin, present in the intact cell, is more susceptible to anesthetic inhibition than is isolated actomyosin. The recent work of Shibata *et al.*,⁹⁸ who have shown that halothane, enflurane, and isoflurane decrease barium ion-induced contracture force and the dynamic stiffness of intact rabbit papillary muscles, may provide support for such a notion.

The possibility also exists that myofibrils are not particularly sensitive to depression by anesthetics, and that the major site(s) for such depression lie elsewhere. Rusy⁹⁹ drew this conclusion from a study of isolated rabbit hearts perfused with a caffeine-containing medium which retarded Ca^{2+} sequestration by the SR.⁶⁹ Three-per-second electrical stimulation caused sustained, tetany-like contractions, due presumably to sustained high sarcoplasmic Ca^{2+} levels, which were not affected by 1.5% (gas phase) halothane. This concentration of halothane depressed contractile force to approximately 20% of control in the same hearts perfused with a medium which did not contain caffeine. Rusy's data however, may not preclude a myofibrillar effect of halothane, as Merin has shown that high Ca^{2+} concentrations are capable of reversing anesthetic depression of actomyosin ATPase.^{92,100} Iwatsuki *et al.*¹⁰¹ observed that thiamylal concentrations as high as 67 mg/l, a concentration which might exist clinically following bolus intravenous injection of the drug, had no effect on force development in glycerinated canine cardiac fibers which were exposed to Ca^{2+} concentrations over the range of 3.0×10^{-7} to 10^{-4} M. Again, while the absence

of any drug effect was striking, Iwatsuki's experiments do not prove conclusively that an effect at the myofibrillar level would not have occurred in a more intact preparation in which phosphorylation was preserved. Additional evidence supporting major extra-myofibrillar sites of anesthetic myocardial depression comes from the work of Su and Kerrick.¹⁰² These investigators used mechanically disrupted rabbit myocardial fibers which they described as functionally skinned, *i.e.*, without functional sarcolemma. Their preparations showed a dose-related increase in tension over the pCa^{2+} range of >9.0 to 3.8 . Exposure to 1% halothane resulted in only a 5% decrease in maximal, Ca^{2+} activated tension. Depression of tension at *submaximal* Ca^{2+} concentrations was not significant until the halothane concentration was greater than 2%. Su and Kerrick compared their observations with those of Brown and Crout,¹⁰³ who showed a 40% depression of intact papillary muscle performance by 1% halothane. They felt that this indicated that the effects of halothane on the contractile proteins could account for only a small percentage of the depression encountered in the intact preparation. In more recent work, Su *et al.* have observed similar (small) effects of pharmacologic doses of enflurane⁸⁶ and isoflurane¹⁰⁴ on maximal Ca^{2+} -induced tension and no significant effect of these anesthetics on submaximal Ca^{2+} -induced tension. Su and Kerrick's experimental preparation is, however, also subject to the problem cited above regarding the phosphorylation state of the contractile proteins. Although this may have been a factor in their not being able to demonstrate much direct myofibrillar depression by halothane, enflurane, and isoflurane, they were, in later experiments,^{85,86,104} able to show moderate to marked depression, by these anesthetics, of the sequestration of Ca^{2+} by the SR, and, secondarily, a depression of contractile force comparable to that seen in the intact myocardium. These later experiments will be discussed in greater detail below. In order to place direct myofibrillar anesthetic depression into proper perspective with other possible sites of anesthetic action, additional work is necessary. Experiments should be carried out in each of several species using intact cell and isolated myofibrillar preparations, wherein both are brought into equilibrium with the same gas phase anesthetic concentrations (several clinically relevant concentrations should be employed), and where the degree of depression of mechanical activity in the intact cell preparations is compared with the depression of mechanical activity or actomyosin ATPase activity in the isolated myofibrillar preparations. Activity at two temperatures, *e.g.*, $25^{\circ}C$ and $37^{\circ}C$, should be examined, since temperature affects both anesthetic solubility¹⁰⁵ and myofilament sensitivity to calcium.¹⁰⁶ In performing the isolated myofibrillar experiments, ion-

ized calcium concentrations should be controlled over a range expected to exist intracellularly. Very importantly, careful attention should be paid to the state of phosphorylation of the isolated myofibrillar preparations and the relative amounts of myosin isozymes should be determined.¹²

SARCOLEMMMA: ELECTRICAL ACTIVITY

The function of the sarcolemma to determine the resting and action potentials and the intracellular ionic environment of the myocardial contractile cell has already been discussed. In 1962, Daniel *et al.*¹⁰⁷ reported that pentobarbital decreased the plateau of the action potential in isolated cardiac muscle. One year later, Levy *et al.*¹⁰⁸ published their study of the effect of cyclopropane on myocardial contractile performance and sarcolemmal electrical activity. They reported marked decreases of contractile tension in rabbit atrial tissue accompanied by only minor changes, in the direction of increased rate of repolarization, of phases 1 and 2 of the action potential. Similar effects on electrical activity in atrial, ventricular, and Purkinje tissue were reported for this agent by Davis *et al.*¹⁰⁹ Summarizing the work of his laboratory on halothane, thiopental, and methoxyflurane, Frederickson¹¹⁰ characterized all of these agents as "uncouplers of excitation-contraction," noting that all were capable of causing marked depression of mechanical activity which was accompanied by only minor changes in action potential configuration, as described above. He noted that increasing $[Ca^{2+}]_o$ reversed contractile depression, but further increased the rate of membrane repolarization. In agreement with Frederickson, Hausworth¹¹¹ reported that halothane caused little alteration of the action potential in rabbit atrial fibers. However, he observed that the action potential duration of sheep Purkinje fibers was appreciably shortened by this agent. An effect of anesthetics on phase 2 (the plateau phase) of the action potential would suggest that it was caused by an alteration of the slow inward Ca^{2+} current, although alteration of an outward K^{+} flux could also be involved.¹¹²

Additional evidence pointing to a major effect of halogenated anesthetics on Ca^{2+} influx has been provided by Lynch *et al.* These workers reported that halothane¹¹³ had very little effect on the maximum rate of rise ($+V_{max}$ of phase 0) of the *normal* action potential in guinea pig papillary muscle, but that, in concentrations of 1% or greater, it caused significant depression of this variable when *slow* action potentials were elicited in this tissue. In addition, they reported that concentrations of this anesthetic greater than 2% caused a significant abbreviation of the plateau of both the normal and the slow action potential.

A myocardial contractile cell which has a normal resting potential of -80 to -90 millivolts is characterized, as noted previously, by an action potential with a fast upstroke, caused by an inward flux of Na^+ and by a plateau phase caused partially by an influx of Ca^{2+} . The inward Na^+ current can be abolished by a long-lasting offset of the resting membrane potential to approximately -50 mv.¹¹⁴ When this is done, for example, by exposing these cells to elevated external K^+ , excitability can be restored by catecholamines⁶³ and action potentials with slow upstrokes (now supported by the slow inward Ca^{2+} current,¹¹⁵ i.e., slow action potentials) can be elicited.¹¹⁴ It is the upstroke (\dot{V}_{\max}) of these slow action potentials that Lynch *et al.* found to be sensitive to halothane. With their additional observation of a depressant effect of higher halothane concentrations on the plateau phase of the action potential, these authors postulated that the negative inotropic effect of halothane could be due, "in part, to decreased Ca^{2+} influx through the slow channel." Since they observed that 0.5% halothane had significant negative inotropic effect without, at the same time, a discernible effect on the slow action potential, they postulated, further, that "additional mechanisms, not involving the slow channel, also participated in the negative inotropic action of halothane." Using similar techniques, Lynch *et al.* reported that enflurane¹¹⁷ did not affect \dot{V}_{\max} of the normal action potential, although the normal action potential duration was decreased by concentrations of 3% or greater of this anesthetic. In contrast, \dot{V}_{\max} of the slow action potential was significantly depressed. As with halothane, contractile force was observed to be even more sensitive to depression than \dot{V}_{\max} of the slow action potential, suggesting to these authors that sites of action in addition to the sarcolemmal slow channel might also underlie enflurane's negative inotropic effect. In later work, Lynch¹¹⁸ found that isoflurane depressed the slow action potential \dot{V}_{\max} to a significant, albeit small, degree, with contractile force being affected to a much greater extent.

Ikemoto¹¹⁹ examined the effect of thiopental on twitch tension and the slow action potential in canine papillary muscles. In his experiments, slow action potentials were induced by caffeine (similar to those induced by catecholamines) following depolarization by high $[\text{K}^+]_o$. Thiopental, 10^{-4} M (26.4 mg/l), depressed both twitch tension and \dot{V}_{\max} of the slow action potential, while the resting membrane potential and resting tension were not affected. Thiopental, 10^{-3} M, nearly abolished both twitch tension and \dot{V}_{\max} . All effects were reversible. Ikemoto *et al.*¹²⁰ made direct measurements of the effect of thiamylal and halothane on the slow inward Ca^{2+} current in isolated rat ventricular myocytes. Both 10^{-4} M thiamylal and 1% halothane caused

the slow action potential duration (measured at 50% repolarization) to decrease to 65% of control. Neither agent altered the resting membrane potential. Using a single electrode voltage clamp technique described by Brennecke and Lindemann,¹²¹ Ikemoto *et al.*¹²⁰ found the slow inward Ca^{2+} current to be depressed to approximately 60% of control by the above indicated doses of both anesthetics. Slow action potentials are also exhibited in SA nodal tissue.¹²² Bosnjak and Kampine¹²³ found that 1 and 2 MAC equivalent halothane, enflurane, and isoflurane significantly depressed the slopes of phases 4 and 0, and decreased the duration of action potentials recorded from single sinoatrial node cells in the isolated, spontaneously beating guinea pig SA node. These effects were partially offset by increasing $[\text{Ca}^{2+}]_o$.

SARCOLEMMMA: ION EXCHANGE

Several investigators have examined the effect of anesthetics on directly measured movements of Ca^{2+} in heart muscle. Porsius and van Zwieten¹²⁴ observed that halothane, in a concentration which decreased the amplitude of contraction of spontaneously beating guinea pig atria by 53%, caused an increase in the rate of ^{45}Ca uptake and in the Ca^{2+} binding capacity of isolated myocardial plasma membranes from this tissue. They interpreted this to mean that a reduced amount of Ca^{2+} was available for excitation-contraction coupling due to halothane's action to accelerate the binding of Ca^{2+} by the plasma membrane. Ohnishi *et al.*¹²⁵ purified a calcium-binding lipoprotein fraction from a canine cardiac plasma membrane preparation previously determined¹²⁶ to be relatively free of SR and mitochondrial membranes, and found that clinically relevant concentrations of halothane, enflurane, and diethyl ether caused increased Ca^{2+} binding by this preparation. They suggested that part of the negative inotropic effect of these anesthetics might be due to a "depression of gated Ca^{2+} transport by increased Ca binding." Additional studies by Ohnishi *et al.* with halothane¹²⁷ demonstrated a linear relationship between decreased contractility and reduction in La^{3+} -displaceable Ca^{2+} in canine trabecular muscle. Using similar methods, Nayler and Szeto¹²⁸ demonstrated that anesthetic doses of pentobarbital also decreased the amount of Ca^{2+} released by La^{3+} from intact cells of canine trabecular muscle. Holding to the assumption that La^{3+} -displaceable Ca^{2+} represents the Ca^{2+} stored superficially by the sarcolemma to be made available for activating contraction during sarcolemmal depolarization,¹²⁹ both Ohnishi *et al.* and Nayler and Szeto postulated that halothane and pentobarbital, by facilitating the binding of Ca^{2+} to membrane phospholipid, made this ion less available for activation of contraction or for release by La^{3+} . Thio-

penthal's effect upon developed tension, action potential, and K^+ and Ca^{2+} exchange in rabbit myocardium was studied by Frankl and Poole-Wilson.¹³⁰ Thiopental at 28 and 227 $\mu M/l$ (7.4 and 60 mg/l) reduced developed tension by 50 and 80%, respectively, regardless of whether the drug was introduced during activity or rest. $^{42}K^+$ exchange was inhibited, an effect thought to be consistent with the observed increase in action potential duration. Both the influx and efflux of Ca^{2+} ($^{45}Ca^{2+}$) were reduced by thiopental. The effect on efflux was immediate and reversible, while the effect on uptake was delayed and not reversible. These workers felt that the observed late decrease in Ca^{2+} uptake was probably *not* related to thiopental's negative inotropic effect. Klaus and Lüllmann¹³¹ also observed that barbiturates and thiobarbiturates inhibited myocardial $^{42}K^+$ exchange. Inhibition was dose-dependent, and occurred only during depolarization. Exchange was inhibited to the same degree in both directions so that no net K^+ shift occurred. Resting K^+ exchange was not affected.

In a study of halothane's action in beating rat heart cells in culture, Malinconico *et al.*¹³² found that this anesthetic caused a dose-related depression of intensity and rate of beating. In the absence of halothane, decrease in $[Ca^{2+}]_o$ below 2.0 mM resulted in a decrease of both rate and intensity. Increasing $[Ca^{2+}]_o$ above 2.0 mM had no effect. In the presence of 1.5% (gas phase) halothane, beating intensity was restored 50% by increasing $[Ca^{2+}]_o$ from 2.0 to 2.5 mM, and was fully restored by 3.0 mM Ca^{2+} . Increasing $[Ca^{2+}]_o$ was without effect on halothane-depressed rate of beating. Release of Ca^{2+} (^{45}Ca) by these cells was not affected by halothane, while uptake was affected in a dose-dependent manner. The effects of halothane to depress Ca^{2+} uptake and beating intensity were closely correlated, and were suggested by Malinconico *et al.* to be causally related.

Taken altogether, these studies demonstrate that anesthetics do alter Ca^{2+} exchange at the level of the sarcolemma. They may also affect sarcolemmal K^+ permeability. An effect on K^+ exchange could well be secondary to a change in $[Ca^{2+}]_i$.^{112,133} The mechanism(s) subserving these alterations of sarcolemmal Ca^{2+} exchange are not well understood. The work of Lynch *et al.*^{113,117,118} and Ikemoto *et al.*¹²⁰ indicates, strongly, an involvement of the slow channel. Preliminary work by Adams and Pruett¹³⁴ suggests that an effect of anesthetics on Na^+/Ca^{2+} exchange may be involved. If such an effect does occur, it could be secondary to an anesthetic-induced alteration of $[Na^+]_i$. The work of Pratila *et al.*,¹³⁵ showing an effect of enflurane and methoxyflurane to reduce post-drive hyperpolarization, suggests that anesthetics may inhibit the activity of the electro-

genic Na/K pump and thereby alter intracellular Na^+ activity. A preliminary study, reported in 1983 by Dedrick and Allen,¹³⁶ described an increase in $[Na^+]_i$ as measured by nuclear magnetic resonance, following halothane administration. A full paper describing this work has not appeared. It should be noted that an increase in $[Na^+]_i$ has been associated with an increase in contractility in studies involving digitalis.⁷⁵ Additional studies of the effects of anesthetics on $[Na^+]_i$ and on Na^+/Ca^{2+} exchange are needed. No studies have been performed to determine whether anesthetics affect the sarcolemmal ATP-dependent Ca^{2+} -pumping system.³⁰ Finally, while anesthetics do inhibit sarcolemmal Ca^{2+} exchange, the contribution of this effect to the production of negative inotropy, *i.e.*, its relative importance amongst other actions of anesthetics, *e.g.*, on SR and on myofibrils, to cause depression of myocardial contractility, is not precisely known. Lynch's cited studies (*vide supra*) and other work, to be discussed, suggest that the relative importance of this effect may vary amongst anesthetics.

SARCOPLASMIC RETICULUM

Anesthetics have been shown to inhibit the uptake of Ca^{2+} by isolated sarcoplasmic reticulum. The early work of Lain *et al.*¹³⁷ suggested that there was little or no correlation between this property of anesthetics and their negative inotropic potencies. For example, these workers found that the concentration of halothane which inhibited Ca^{2+} uptake in isolated canine SR was approximately 10 times higher than that required to inhibit contractility in isolated papillary muscle. Ether, which clearly exhibited negative inotropism, was without effect on SR Ca^{2+} uptake. In contrast to this, chloroform was found to be equipotent as regards depression of these variables. Lain *et al.* found that Ca^{2+} uptake and Ca^{2+} -dependent ATPase activity were affected equally by chloroform and halothane, and interpreted this to mean that these agents did not uncouple Ca^{2+} transport from ATP hydrolysis. Pentobarbital, on the other hand, did exhibit uncoupling. In a more recent publication, Malinconico and McCarl¹³⁸ pointed out that the experiments of Lain *et al.* were performed at a non-physiological concentration of Ca^{2+} , *i.e.*, $[Ca^{2+}] > 0.1$ mM. Using more physiological Ca^{2+} concentrations of 0.4 to 20 μM , Malinconico and McCarl demonstrated that clinically relevant halothane levels depressed Ca^{2+} -activated ATPase activity in isolated bovine SR at a pH of 7.0. They found that increasing the Ca^{2+} concentration of the external medium antagonized this depression, and stated that halothane inhibition of Ca^{2+} -ATPase appeared to be competitive in nature (although possibly not pharmacologically competitive, since they could

provide no evidence that halothane and Ca^{2+} acted at the same receptor). Their work led them to state that "halothane depression of SR function may in part explain the ability of halothane to depress myocardial function." Blanck and Thompson¹³⁹ found that the effect of halothane on the *in vitro* uptake of Ca^{2+} by isolated canine myocardial SR was dependent on both the H^+ and ATP concentration. At a normal ATP concentration (5 mM) and a pH of 7.3, 1.75% halothane was found not to affect the steady-state level of Ca^{2+} in the SR. At a pH of 6.9, depression of Ca^{2+} uptake was observed. At a low (0.5 mM) ATP concentration, Ca^{2+} uptake was increased 800% by the anesthetic. Similar effects were observed for enflurane and isoflurane.¹⁴⁰ Blanck and Thompson concluded that the *in vitro* depressant effect of these anesthetics on the SR, which they observed at a pH of 6.9, was unlikely to be of importance as a mechanism underlying a negative inotropic effect in the normal heart "in which the ATP concentration is approximately 5 mM and the pH is approximately 7.4."¹³⁹ The disparity between the conclusions of Blanck and Thompson and Malinconico and McCarl depends primarily on the true intracellular pH in normal heart. Current *in vivo* measurements of cardiac intracellular pH indicate that it is in the range of 7.0–7.2.¹⁴¹ In more recent work, Casella *et al.*¹⁴² have found that, while halothane, enflurane, and isoflurane reverse the depressant effect of alkaline pH on the rate of Ca^{2+} uptake by isolated SR (thus increasing the rate of Ca^{2+} uptake), the total capacity of the SR for Ca^{2+} is decreased by these anesthetics at all pHs over the range 6.6–7.6. It appears, then, that the preponderance of evidence obtained from *in vitro* studies of the effect of anesthetics on isolated SR suggests that inhibition of Ca^{2+} sequestration (and, secondarily, a decrease in the amount of Ca^{2+} available for internal release by SR) could be responsible, in part, for the negative inotropic action of these agents.

Additional studies of the alteration of SR function by anesthetics have been performed by Su *et al.*, who examined the effect of halothane,⁸⁵ enflurane,⁸⁶ and isoflurane¹⁰⁴ on SR Ca^{2+} uptake capacity and SR Ca^{2+} release using an experimental preparation in which this organelle remained *in situ*. Maximal Ca^{2+} release from Ca^{2+} -loaded SR in functionally skinned rabbit myocardial fibers was elicited by addition of caffeine (25 mM) to the bathing medium.^{9,51} The amount of Ca^{2+} released was evaluated by measuring the elicited tension transient. Anesthetic effects on release were determined by exposing the preparation to the anesthetic *only* during the release phase, while effects on SR Ca^{2+} uptake were evaluated by restricting anesthetic exposure to the Ca^{2+} loading phase. Note that Su *et al.* studied SR Ca^{2+} uptake capacity, not the kinetics of Ca^{2+} uptake. In these

studies, a pH of 7.0 and a temperature of 20° C was maintained in all media. Azide was used in some studies to prevent Ca^{2+} uptake by mitochondria in order to demonstrate that mitochondrial Ca^{2+} release did not play a role in causing the tension transients. For 0.5–3.0% halothane, depression of SR Ca^{2+} uptake varied, dose-dependently, from approximately 20–90%. For enflurane (2.5–7.5%), depression ranged from 30–85%, while, for isoflurane (3–4%), depression, though significant, was not as marked, amounting to 15–25%. No statistically significant effect on maximal Ca^{2+} release was observed for halothane, enflurane, or isoflurane. Overall anesthetic effect on uptake and release (anesthetic present during both phases) followed a pattern indistinguishable from the effect on Ca^{2+} uptake alone. Su *et al.* speculated that anesthetic-induced myocardial depression could be caused by a direct effect of anesthetics to inhibit SR Ca^{2+} uptake. Interestingly, Su and Kerrick⁸⁵ saw a suggestion (although not statistically significant) of enhancement of maximal Ca^{2+} release when exposure to halothane was restricted to the release phase. Since they reasoned that maximal (essentially total) Ca^{2+} release by 25 mM caffeine would leave little room for enhancement by the anesthetic, they studied submaximal release using 2 mM caffeine. Halothane markedly and significantly enhanced caffeine-induced submaximal Ca^{2+} release. At a concentration of 1%, it caused a 70% increase in the observed tension transient. Citing Endo's¹⁴³ speculation that Ca^{2+} -induced release of Ca^{2+} and caffeine-induced Ca^{2+} release were basically related, Su and Kerrick⁸⁵ postulated that, since halothane enhanced caffeine-induced release, it might also enhance Ca^{2+} -induced release of Ca^{2+} . They postulated, further, that, if this were the case, the observed "decreased ability of the internal store (SR) to sequester Ca^{2+} in the presence of halothane (could), in part, be explained by a decrease in the equilibrium ratio between the rate of Ca^{2+} uptake and release." It should be noted that, under the condition of their experiment, these authors were not able to determine whether halothane alone caused Ca^{2+} release from the SR, nor did they study Ca^{2+} -induced Ca^{2+} release. Enhancement of caffeine-induced submaximal Ca^{2+} release was also demonstrated for enflurane⁸⁶ and isoflurane.¹⁰⁴

Su *et al.* concluded, from these studies, that depression of net SR Ca^{2+} uptake could contribute to the negative inotropic effect of all of these anesthetics, amounting to a major factor for halothane and enflurane, and a significant, but relatively less important one, for isoflurane. While these authors do provide evidence, supportive of that obtained from studies of isolated SR, that anesthetics inhibit SR Ca^{2+} sequestration, their observations do not (as they admit) document whether such inhibition of sequestration occurs primarily through a

direct inhibition of Ca^{2+} uptake (as suggested by *in vitro* studies¹³⁸ showing depression of Ca^{2+} activated SR ATPase activity), or through an increase in the rate of Ca^{2+} leak from this organelle, or, possibly, both. Their speculation regarding a possible role for anesthetic enhancement of SR Ca^{2+} release needs further study. In particular, caffeine-induced Ca^{2+} release (the type of release upon which their speculation was based) may bear only superficial resemblance to the physiological mechanism(s) responsible for SR Ca^{2+} release. For example, caffeine-induced Ca^{2+} release does not share the refractory period characteristic of Ca^{2+} -induced release of Ca^{2+} ,¹⁰⁶ and, in addition, it has been shown to occur after physiological SR Ca^{2+} release has been blocked by ryanodine.¹⁴⁴ A possibility to be considered is that halothane increases the rate of Ca^{2+} leak from the SR during diastolic intervals, resulting in the depletion of Ca^{2+} that is to be released from the SR upon depolarization of the sarcolemma. Studies of the effect of anesthetics on Ca^{2+} -induced release of Ca^{2+} by the SR, yet to be performed, should provide additional, very useful information. The link coupling sarcolemmal depolarization to SR Ca^{2+} release is, perhaps, the least well-understood aspect of electromechanical coupling.³⁸ Thus, it is not surprising that our understanding of related anesthetic action(s) is incomplete.

ISOLATED INTACT MYOCARDIUM

A number of studies have been designed with the aim of determining, in isolated intact heart muscle, the relative roles played by anesthetic inhibition of the transsarcolemmal and sarcoplasmic reticular sources of activator Ca^{2+} in depressing myocardial contractility. Advantages of this type of study include the possibility of examining anesthetic effects on the subcellular elements which control the myocardium's contractile state, while these elements are in their normal environment. Disadvantages include the fact that intracellular myoplasmic Ca^{2+} levels are not controlled or precisely known, and that interpretation of the information obtained is dependent on the use of one of several proposed models of electromechanical coupling, none of which is yet universally accepted. Such information can, however, be usefully compared with that derived from studies of isolated organelles to provide a better understanding of anesthetic-induced myocardial negative inotropism.

Bosnjak and Kampine¹⁴⁵ have used the photoprotein, aequorin, to examine the effect of halothane on intracellular Ca^{2+} transients in isolated cat papillary muscles. They have compared these measurements with simultaneous measurements of transmembrane potentials and isometric developed tension. Halothane caused a dose-

related decrease in developed tension and transient $[\text{Ca}^{2+}]_i$ peak. The muscles exhibited positive staircase (both force and Ca^{2+} transient) as the pacing rate was increased (60, 75, 100, and 150 beats per minute) during the control condition and during exposure to 1.2 and 2.4% halothane. When verapamil, 2 μM , was added, staircase (both force and Ca^{2+} transient) became negative under all conditions, probably due to the frequency dependent Ca^{2+} channel blockade caused by verapamil (*i.e.*, increasing block with increasing rate of stimulation).¹⁴⁶ Halothane caused a slight decrease in the duration of the action potential, and verapamil caused a small increase. Halothane, added in the presence of verapamil, caused a further increase in action potential duration. No change in the resting membrane potential or in the action potential amplitude was noted. The authors observed, at the higher pacing rates, that "halothane had a greater effect on force compared with intracellular Ca^{2+} " and that "increasing the halothane concentration from 0.18 to 0.33 mM (1.2 to 2.0% gas phase in Bosnjak's and Kampine's experimental set up) substantially decreased force while the aequorin signal was abbreviated to a lesser degree." They suggested, because of these unequal effects, that halothane decreased myofilament sensitivity to Ca^{2+} , although they stated this with reservation, since "the aequorin signal may not accurately measure the Ca^{2+} concentration immediately surrounding the myofilaments" and since "neither the contractile force nor the Ca^{2+} transient signal is likely to be linearly related to the (intracellular) concentration of Ca^{2+} under these conditions." Based on the results of this study and their previous study of the SA node (ref. 123, *vide supra*) and on the results of the work of Lynch,¹¹³ the authors conclude that "halothane reduces the influx of extracellular Ca^{2+} " and that "this decrease in Ca^{2+} influx is likely to be responsible for a smaller release of Ca^{2+} by the sarcoplasmic reticulum resulting in a depressed intracellular $[\text{Ca}^{2+}]_i$." This study is important, since direct measurements appear to indicate that intracellular myoplasmic activator Ca^{2+} levels are decreased by halothane. However, Bosnjak's and Kampine's conclusions cannot be accepted unequivocally. The muscles they used were relatively thick (mean diameter of 1.3 mm), and, because of this, may have been hypoxic.¹⁴⁷ The fact that these authors report maximum developed tensions of only about 200 mg/mm^2 (an order of magnitude below expected) indicates that this may be true. Also, as they mention, halothane might directly influence Ca^{2+} -induced aequorin luminescence.^{148,149} They offer data from *in vitro* work of their own, showing that aequorin luminescence, induced by 10 mM Ca^{2+} , is not affected by the concentrations of halothane to which the muscles were exposed. However, this data may not rule out an effect intracel-

lularly, where aequorin luminescence is induced by much smaller (micromolar) concentrations of Ca^{2+} .

Using rested-state contractions (*vide supra*) and steady-state contractions at frequencies of 0.1–3.0 Hz, in isolated intact guinea pig papillary muscles, Lynch¹¹⁸ examined the effects of halothane and isoflurane on normal and slow action potentials and upon the mechanical responses accompanying these. For normal action potentials, isoflurane (1.3 and 2.5%) depressed peak developed tension significantly less at high stimulation frequencies than did equivalent doses of halothane (0.75 and 1.5%). At the low stimulation frequency of 0.3 Hz, developed tension was late peaking, and was depressed equally by both anesthetics. When rested-state and low-frequency contractions were elicited in a low-sodium extracellular environment, which presumably enhanced SR Ca^{2+} sequestration by inhibiting $\text{Na}^+/\text{Ca}^{2+}$ exchange,^{44,46} tension was early peaking and was depressed significantly less by isoflurane than by halothane. For slow action potentials, elicited by high extracellular K^+ and 0.1 μM isoproterenol (*vide supra*) tension exhibited both early and late peaking phases. Again, late peaking tension was depressed equally by both halothane and isoflurane, while early peaking tension was depressed significantly less by isoflurane than by halothane. Depression of \dot{V}_{max} , the rate of upstroke of the slow action potential, was 42% less with isoflurane than with an equivalent dose of halothane, while isoflurane caused a greater depression of contractile tension than halothane. Because "isoflurane altered the pattern of tension development in a different manner than halothane," Lynch suggested that these anesthetics depressed myocardial contractility by different mechanisms. He ascribed late peaking tension to activation by Ca^{2+} , which enters the cell during the action potential, thence being sequestered and released by the SR during the same beat, as proposed by Reiter *et al.*⁴⁶ Thus, depression of late peaking tension is suggested by Lynch to be due to anesthetic inhibition of SR function, an action possessed by both halothane and isoflurane. Since isoflurane depressed \dot{V}_{max} of the slow action potential (a measure of the Ca^{2+} -mediated slow inward current) significantly less than halothane, and approximately five-fold less than it depressed late peaking tension, Lynch proposed that "decreased Ca^{2+} entry contributed little to the contractile depression produced by isoflurane" and that "an alteration in SR uptake and/or release would explain the dramatic depression of late peaking tension" caused by this anesthetic. Halothane's negative inotropic effect would be mediated by depression of both Ca^{2+} entry and SR function. While Lynch did not suggest a source of activator Ca^{2+} for early peaking tension, other workers^{46,66,150–153} have postulated that it is Ca^{2+} which has accumulated in the SR

during previous beats for immediate release following stimulation. In favor of this, these workers have shown that early peaking tension is inhibited by typical inhibitors of SR function, such as ryanodine and the methylxanthines. If early peaking tension is mediated by SR release of Ca^{2+} , Lynch's finding that early peaking tension is only minimally affected by isoflurane does not support his postulation that this anesthetic's major action in regard to depression of contractility is one of SR inhibition. Nor is his postulation supported by the observation of Su and Bell,¹⁰⁴ mentioned earlier in this review, that isoflurane suppression of SR function is small compared to the suppression caused by equivalent doses of halothane. Lynch has also provided some data for enflurane¹¹⁸ which indicates that, although it closely resembles isoflurane structurally, its actions in guinea pig papillary muscle are very much more like those of halothane.

Komai and Rusy¹⁵⁴ examined rested state and potentiated state contractions in isolated, intact rabbit papillary muscles in an attempt to determine the extent to which actions of halothane in the sarcolemma and the SR were responsible for the anesthetic's negative inotropic effect. The rationale for these experiments was based on the previous work of others which indicated that rested-state contractions depended almost exclusively upon transsarcolemmally derived Ca^{2+} for their activation,^{44,53} while potentiated-state contractions were activated largely by Ca^{2+} derived from internal SR release.^{28,54} Rested-state contractions were elicited by a single stimulus delivered after 20 min of rest, while potentiated-state contractions were produced by paired-pulse (post-extrasystolic) stimulation. Halothane (0.6 and 1.0%, gas phase) caused approximately equal inhibition of rested-state and potentiated-state contractions. In addition, halothane accelerated the rate of decay of the potentiated state, which was taken to suggest that it decreased the ability of the SR to retain Ca^{2+} during rest. It was concluded that halothane's negative inotropic action was caused by a combination of quantitatively similar inhibitory effects on the transsarcolemmal and intracellular (SR) sources of activator Ca^{2+} .

Using similar methods, Komai and Rusy¹⁵⁵ compared the actions of halothane (0.6%) and isoflurane (1.4%) (anesthetic concentrations causing equal depression of contractile force at a stimulation frequency of 0.05 Hz) to those of Ni^{2+} , an inhibitor of the slow inward Ca^{2+} current,⁴⁴ and ryanodine, a selective inhibitor of SR function.⁵⁴ Confirming Lynch's earlier observation (*ref.* 118, *vide supra*), they found that the negative inotropic effect of isoflurane was significantly less than that of halothane at high, but not at low, steady-state stimulation frequencies. In the presence of isoproterenol, however, isoflurane caused significantly less depression than

halothane, even at low stimulation frequencies. Increasing the stimulation frequency in the presence of isoproterenol *completely* reversed (*i.e.*, to values of developed force measured in the presence of isoproterenol alone) the negative inotropic effect of isoflurane, but not that of halothane. In addition, these investigators found that the inhibitory effect of isoflurane on potentiated state (post-rest) contractions was significantly less than that on steady-state (2.0 Hz) contractions. The effect of halothane is opposite in this regard.¹⁵⁶ Isoflurane's differential effect on steady-state and potentiated-state contractions resembled that of Ni^{2+} (an inhibitor of the slow inward current without frequency dependent effects), and was opposite to the effect of ryanodine (a selective inhibitor of SR function). It should be noted here that, although it is generally agreed that ryanodine specifically inhibits SR function, the mechanism whereby the drug accomplishes this is incompletely understood. It apparently inhibits Ca^{2+} release from a subfraction derived from *junctional* SR, but not from longitudinal SR.^{157,158} While reduction of SR Ca^{2+} release has been argued to occur secondary to a direct inhibitory effect on the *release* process,¹⁵⁹ it has also been stated to occur secondary to accelerated depletion of stored Ca^{2+} .¹⁶⁰ Nevertheless, it is apparent that the availability of activator Ca^{2+} is reduced by ryanodine, regardless of the underlying mechanism. Komai and Rusy concluded that isoflurane, like halothane, inhibited the influx of extracellular Ca^{2+} , but, unlike halothane, had only a minimal effect on the availability of Ca^{2+} stored in and released from the SR. Isoflurane's, but not halothane's, negative inotropic effect was reversed by conditions which increase transsarcolemmal Ca^{2+} influx. While Komai and Rusy agree with Lynch that the mechanisms subserving the negative inotropic effects of halothane and isoflurane are different, their conclusions regarding mechanism are essentially opposite. A major reason for this discrepancy rests in the fact that the observations provided by these studies depend, for their interpretation, on the use of models of electromechanical coupling (*vide supra*), none of which is as yet universally accepted. Specifically, in this instance, Komai and Rusy interpret SR Ca^{2+} as that which is functionally inhibited by ryanodine or the methylxanthines, while Lynch, as mentioned above, interprets SR function according to the model proposed by Reiter *et al* (ref. 46, *vide supra*). As mentioned earlier the link between membrane depolarization and internal release of Ca^{2+} is poorly understood. Perhaps there is more than one source of Ca^{2+} available for internal release, each with a different triggering mechanism. As more is learned about these phenomenon, the apparent controversy between Lynch and Komai and Rusy as regards isoflurane's mechanism of action is likely to be resolved.

Davies and McCans¹⁶¹ examined the effects of barbiturate anesthetics and ketamine on the force-frequency (Bowditch staircase) relation of isolated perfused rabbit hearts. The hearts were perfused with Krebs solution and held at a temperature of 37° C. A strain gauge was sutured to the free wall of the left ventricle, allowing developed force and its derivative (dF/dt) to be measured. Hearts were paced at 1.0, 2.0, and 2.5 Hz. Ketamine-HCl (0.8 mM, 219 mg/l), pentobarbital sodium (1.2 mM, 298 mg/l), thiopental sodium (0.8 mM, 210 mg/l), and methohexital sodium (0.7 mM, 199 mg/l) each caused approximately 80% depression of dF/dt . In the absence of anesthetic, staircase was positive. At 2.5 Hz, dF/dt was 140% of the value at 1.0 Hz. Staircase remained positive for all doses of pentobarbital. Ketamine, thiopental, and methohexital exerted their greatest depressant effect at high pacing rates and at high dose, where they caused staircase to become negative, an effect which was enhanced by increasing the concentration of Ca^{2+} in the perfusate. The authors proposed that all four drugs acted to inhibit the transsarcolemmal influx of Ca^{2+} and to depress contractile force. However, as Davies and McCans state, "simple inhibition of Ca^{2+} influx is not sufficient to reverse staircase." They reason: La^{3+} ¹⁶² blocks Ca^{2+} influx by displacing Ca^{2+} from superficial binding sites in the sarcolemma. While this ion depresses contractile force, it does not reverse staircase.¹⁴⁶ Verapamil and D600, on the other hand, act directly on the Ca^{2+} channel to alter its kinetics, *i.e.*, they cause a frequency-dependent blockade of the Ca^{2+} channel,^{146,163,164} and are capable of reversing staircase.^{146,165} Pentobarbital has been reported to reduce the amount of Ca^{2+} displaced from the sarcolemma by La^{3+} .¹²⁸ Davies and McCans thus suggest that ketamine, thiopental, and methohexital, because of their ability to reverse staircase, have a rate-dependent action of sarcolemmal Ca^{2+} channels similar to that of verapamil and D600. It should be noted that staircase reversal by these anesthetics occurred only at drug concentrations which were very high compared to those likely to be encountered clinically.

Komai and Rusy¹⁵⁶ compared the actions of halothane (0.5, 1.0, and 1.5%) and thiopental (10, 20, and 30 mg/l), in isolated, intact rabbit papillary muscles. Differences in the action of these anesthetics on staircase and potentiated state contractions were noted. A positive staircase was produced by increasing the stimulation frequency from 1.0 to 2.0 Hz. Recall that Langer *et al.* (ref. 56, *vide supra*), have provided evidence that the increased Ca^{2+} made available for staircase comes from the sarcolemma. Additionally, Sutko *et al.*⁵⁴ have shown staircase to be relatively insensitive to ryanodine (*vide supra*), indicating that the SR may not be involved in its production. Potentiated state contractions, on the

other hand, are exquisitely sensitive to ryanodine,⁵⁴ and are, therefore, dependent upon Ca^{2+} derived from the SR for their activation. In agreement with Davies and McCans,¹⁶¹ the negative inotropic effect of thiopental was more prominent at high stimulation frequencies. Thiopental, therefore, inhibited staircase, *i.e.*, decreased the percent change in force accompanying staircase. Halothane had the opposite effect, *i.e.*, it had a greater depressant effect at low stimulation frequencies and caused staircase to be steeper. Thiopental inhibited potentiated state contractions significantly less than steady-state contractions. Halothane, on the other hand, inhibited potentiated state contractions significantly more than steady-state contractions. Thiopental shifted the curve of post-rest potentiated force *versus* length of rest period to the right, while halothane shifted this curve to the left. Edman and Jóhannsson⁴³ have shown that post-rest potentiation requires an optimum length of rest period and have interpreted this to mean that, during rest, the Ca^{2+} which is ultimately responsible for potentiation is transported, in a time-dependent manner, from sites of SR uptake to sites of release. Using this model for post-rest potentiation, Komai and Rusy suggest that thiopental slows intra-SR Ca^{2+} transport, while halothane hastens it (although only slightly). An alternatively proposed model,⁴⁷ kinetically indistinguishable from Edman and Jóhannsson's, incorporates a single Ca^{2+} uptake/release site, and suggests that a time-dependent recovery of activation of Ca^{2+} release channels in the SR is responsible for the optimum rest period.

In summary, Komai and Rusy state that both anesthetics reduce the transsarcolemmal influx of activator Ca^{2+} . While thiopental slows intra-SR transport of Ca^{2+} (or increases the time for reactivation of Ca^{2+} release channels), thereby increasing the time needed for development of potentiation, it does not appreciably reduce the amount of SR Ca^{2+} available for release, *i.e.*, its effect on the force of potentiated state contractions is relatively small. The major action responsible for thiopental's negative inotropic effect is inhibition of transsarcolemmal Ca^{2+} influx. For halothane, on the other hand, inhibitory effects in both the sarcolemma and the SR act, importantly, to cause depression of myocardial contractility.

Komai *et al.*¹⁶⁶ studied the negative inotropic effect of etomidate, 2.0 and 4.0 mg/l, using steady state and post-rest potentiated state contractions in rabbit papillary muscles. Etomidate's effect was most pronounced at low stimulation frequencies (note that the opposite is true for thiopental). Also, unlike thiopental, etomidate had virtually no effect on the length of the rest period needed for optimum post-rest potentiation, but, like thiopental, etomidate's effect on the magnitude of po-

tentiated state contractions was small compared to its steady-state effect. The negative inotropic effect of etomidate, both 2.0 and 4.0 mg/l, could be reversed by increasing extracellular Ca^{2+} . These authors observed, as did Kissin *et al.*,¹⁶⁷ that the negative inotropic effect of etomidate was relatively small. They concluded that this action was mediated primarily by inhibition of Ca^{2+} influx with very little effect on the availability of intracellular (SR) Ca^{2+} .

Summary

The bulk of experimental evidence indicates that anesthetics do not produce their negative inotropic effect *via* an inhibitory action on mitochondrial electron transport. Anesthetics decrease energy need, rather than energy production. Anesthetics also decrease the rate of sequestration of Ca^{2+} by mitochondria, but, again, this appears not to be an important cause of reduced myocardial contractility.

The role played by direct anesthetic depression of the myofibrils in reducing contractility is uncertain. Most experimental evidence now available suggests that significant myofibrillar depression, measured in terms of inhibition of actomyosin ATPase activity or inhibition of force production, occurs only at anesthetic concentrations which are high compared to concentrations employed clinically. This would seem to indicate that the myofibrils are not an important target for anesthetics in regard to the production of depressed myocardial contractility. However, the experimental act of removing myofibrils from their intracellular environment, or of removing the sarcolemma or making it hyperpermeable, appears to prevent some regulatory myofibrillar phosphorylation reactions from taking place. As stated by Winegrad,¹² "certain forms of regulation of the cardiac myofibril are fragile and can be seen only when cellular constituents and structure are maintained." It is possible that this type of regulation is susceptible to inhibition by anesthetics. Methods for preserving this regulation are available,¹² and will need to be employed before a depressant action of anesthetics on the myofibril can be definitely dismissed as a significant cause of the inhibition of cardiac contractility. A single study, of intracellular Ca^{2+} levels in the intact cell (where myofibrillar regulation was presumably preserved), has indicated that halothane may decrease myofilament Ca^{2+} sensitivity. However, for reasons stated above, this study cannot be taken as unequivocal proof of such an action.

Despite the fact that the normal action potential is little affected by anesthetics, the sarcolemma appears to play a pivotal role in the production of anesthetic-induced contractile depression. Significant depression of

the rate of upstroke of the slow (Ca^{2+} -mediated) action potential by clinical concentrations of both inhalation and intravenous anesthetics has been demonstrated by several workers. This has been interpreted to mean that anesthetics inhibit the influx of Ca^{2+} through the slow channel, and such has been confirmed (to date, for halothane and thiamylal) by direct measurement of the slow inward Ca^{2+} current using a voltage clamp technique. In addition to inhibition of the slow channel, anesthetics (both volatile and intravenous) have been shown to increase Ca^{2+} binding by the plasma membrane. This is believed to decrease the amount of Ca^{2+} available for contractile activation. According to our present understanding of electromechanical coupling, inhibition of the sarcolemma in regard to its function as a source of activator Ca^{2+} could result in depression of contractility by: 1) decreasing the portion of transsarcolemmally derived Ca^{2+} which may participate directly in myofibrillar contractile activation, 2) decreasing the Ca^{2+} which is made available for storage in, and later release by, the SR, or 3) decreasing the transsarcolemmally derived Ca^{2+} which has been postulated to trigger the internal release of Ca^{2+} from the SR. Voltage clamp studies of the effect of enflurane, isoflurane, and the intravenous anesthetics (in addition to thiamylal) on the slow inward Ca^{2+} current are needed, as are studies of anesthetic effects on $\text{Na}^+/\text{Ca}^{2+}$ exchange, on intracellular $[\text{Na}^+]$, and on sarcolemmal Ca^{2+} -ATPase activity. Studies of the effect of anesthetics on myocardial Ca^{2+} influx, using a newly developed method^{28,168} which monitors transient depletion of extracellular Ca^{2+} with Ca^{2+} -selective microelectrodes in a relatively intact muscle preparation, would provide additional, useful information.

A role for the participation of the sarcoplasmic reticulum in the production of anesthetic-induced negative inotropism also appears to exist. *In vitro* studies of this organelle indicate that SR Ca^{2+} -ATPase activity (and, presumably, Ca^{2+} uptake) is depressed in a dose-related fashion by anesthetics. Anesthetic effects on the rate of Ca^{2+} uptake by the SR have been found to be dependent on both pH and ATP concentration. However, the total capacity of the SR for Ca^{2+} appears to be decreased by anesthetics under all conditions, and this has been interpreted to mean that less Ca^{2+} is available for internal release in the presence of these agents. Studies in intact tissue tend to confirm this impression, especially for halothane and enflurane. Although the data from *in vitro* studies are insufficient to determine whether there are quantitative differences between anesthetics concerning their action in SR, studies in more intact preparations infer that such differences exist. The results of these studies have been interpreted by some (albeit not all) investigators to suggest that isoflu-

rane exerts less of a depressant effect on SR function than either halothane or enflurane. Likewise, the intravenous anesthetics which have been examined appear to depress SR function less than the inhalation agents in regard to decreasing the amount of Ca^{2+} which is available for internal release. Data from certain of these investigations indicates, also, that reversal of contractile depression by positive inotropic agents is more complete for those anesthetics that minimally affect the SR. As indicated earlier, the extent to which inhibition of sequestration of Ca^{2+} by anesthetics is caused by direct inhibition of Ca^{2+} uptake or by an increase in the rate of Ca^{2+} leak from this organelle is not known. That the latter mechanism is important for halothane is suggested by studies in intact tissue, which show an increased rate of decay of the potentiated state in the presence of the anesthetic. Data concerning this effect is not available for other anesthetics, and should be obtained. Also, *in vitro* studies of the effect of anesthetics on the rate of Ca^{2+} leak from Ca^{2+} -loaded isolated SR should be performed. Finally, no *physiological* mechanism presently proposed to be responsible for the internal release of Ca^{2+} from the SR has been directly examined for possible influence by anesthetics. In this regard, the effect of anesthetics on Ca^{2+} -induced release of Ca^{2+} should be evaluated, both *in vitro* and in skinned fiber preparations. Similarly, studies of the effect of anesthetics on IP_3 -induced internal release of Ca^{2+} should also be performed.

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