Slow Inward Current and Contraction of Sheep Cardiac Purkinje Fibers

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ABSTRACT A "slow" inward current ($I_s$) has been identified in ventricular muscle and Purkinje fibers of several mammalian species. The two-microelectrode voltage clamp technique is used to examine some of the relationships between $I_s$ and contraction of the sheep cardiac Purkinje fiber. "Tails" of inward current occurring on repolarization and extrapolation of $I_s$ recovery each show that the $I_s$ system may not inactivate completely during prolonged depolarization. The rate of recovery of $I_s$ after a depolarization is slow, and when a train of 300-ms clamps (frequency 1 s$^{-1}$) is begun after a rest, $I_s$ is larger for the first clamp than it is for succeeding clamps. For the first clamp after a rest, the thresholds for $I_s$ and tension are the same and there is a direct correlation between peak tension and peak $I_s$ for clamp voltages between threshold and $-40$ mV. After a clamp, however, the ability to contract recovers much more slowly than does $I_s$. Therefore, since $I_s$ may occur under certain conditions without tension, the relationship between $I_s$ and tension must be indirect. Calcium entering the cell via this current may replenish or augment an intracellular calcium pool.

INTRODUCTION

An inward current with slow kinetics ($I_s$) has been identified in cardiac Purkinje fibers under voltage clamp conditions (Reuter, 1967, 1968; Vitek and Trautwein, 1971) and in ventricular muscle of several species (see reviews by Bassingthwaighte and Reuter, 1972; Reuter, 1973; and Trautwein, 1973). Calcium and sodium ions may both contribute to the slow inward current in Purkinje fibers, but in spite of the possibility that sodium ions may carry part of the charge, the conductance system responsible for $I_s$ appears to be separate from that which is responsible for the fast inward sodium current ($I_{Na}$).

Because calcium ions carry much of the charge responsible for $I_s$, this current could play an important role in excitation-contraction coupling. In ventricular muscle, $I_s$ appears to allow calcium accumulation in intracellular stores (Bassingthwaighte and Reuter, 1972). The slow inward current may
therefore be an important determinant of contraction, and it may be especially important in explaining the dependence of cardiac contraction on the rate and rhythm of stimulation.

The relationship between \( I_s \) and the contraction of Purkinje fibers has not been examined, partly because of difficulties in determining the characteristics of the current in the presence of other overlapping currents. The available data on \( I_s \) (Reuter, 1967, 1968; Vitek and Trautwein, 1971) and on the contractile behavior of Purkinje tissue (Fozzard and Hellam, 1968; Gibbons and Fozzard, 1971, 1975) suggest that there may be interesting correlations between the two.

METHODS

Equipment and Procedure

These experiments were performed with the same equipment and under the same conditions described in the preceding paper (Gibbons and Fozzard, 1975). In particular, Tyrode's solution with 2.7 mM calcium was used throughout at temperatures between 35 and 37°C. The full composition of the Tyrode's solution was (in mM): NaCl, 137; KCl, 5.37; MgCl₂, 1.05; NaHCO₃, 13.5; NaH₂PO₄, 2.4; CaCl₂, 2.7; dextrose, 11.1. It was saturated with a mixture of 95% O₂ and 5% CO₂.

Current Identification and Measurement

The inward current to be examined has been referred to by various authors as the "slow" inward current, "secondary" inward current, and in the case of ventricular muscle as the "calcium" inward current. None of these names is truly appropriate here, for the current is slow only in the sense that its kinetics are slower than those of the sodium inward current, it is secondary only if a sodium inward current precedes it, and sodium ions may carry part of the charge responsible for the current in Purkinje fibers. Slow inward current appears to be the choice of most authors, and we will use either this or \( I_s \) to refer to this current.

Without ion substitution or the addition of pharmacological blocking agents, the slow inward current can be examined only in the voltage range between the holding potential and \(-20\) mV. At the more negative voltages in this range, the slow inward current is usually a net inward current. After 200–250 ms, net current is almost always outward, and constant. At less negative voltages, net current (after the inactivation of \( I_{Na} \)) is frequently outward throughout the clamp, and the slow inward current is identified as an inwardly directed deflection of the current trace.

The constant outward current reached after 200–250 ms allows the slow inward current to be measured as the difference between the steady outward current at the end of the clamp and the peak of the deflection attributed to \( I_s \) (Fig. 1).

Limitations of the Measurements

In making the measurements in this way we tacitly assume that the current trace becomes flat because \( I_s \) has been completely inactivated. This is not necessarily so.
There might be a significant inward current at the point where the base line is taken if, for example, inward current has declined to a steady level without completely shutting off. Our experiments suggest that this is a possibility. The method used here may therefore underestimate the peak current in the voltage range where measurements can be made, and it does not allow any measurements at voltages positive to -20 mV because the large outward (chloride) current which appears at voltages positive to -20 mV (Dudel et al., 1967; Fozzard and Hiraoka, 1973) obscures any small inward current which may be present. As argued by New and Trautwein (1972), however, the method chosen gives a more accurate estimate than would be obtained using the zero current line as a reference.

Another measure of $I_s$, perhaps the integral of current vs. time, might result in somewhat different relationships than those presented here. Until the role of this current is better understood, however, the method used at least offers simplicity, and the results indicate a number of interesting relationships between the slow inward current and tension.

RESULTS

Fig. 1 is a typical record showing the slow inward current and its relation to contraction. In this example, $I_s$ peaks about 20 ms after the clamp depolarization. Less than 200 ms after the beginning of the clamp, the current is outward and constant. Upon repolarization to the holding voltage, there is a very small "tail" of inward current. Time to peak tension, measured from the beginning of depolarization, is 100 ms. At this voltage, there is no measurable steady tension after the phasic response has declined. Such steady responses begin to be seen at voltages positive to -20 mV (Gibbons and Fozzard, 1971) where measurement of the slow inward current is impossible, so we could only examine the relation between $I_s$ and the phasic part of contraction.

Vitek and Trautwein (1971) and New and Trautwein (1972) have examined the time-course of the conductance change responsible for $I_s$ by giving a

![Figure 1](image-url)

**Figure 1.** General characteristics of the slow inward current and its relation to contraction. Membrane voltage (V), membrane current (I), and tension (P) are shown for a 500-ms clamp from a holding voltage of -79 mV to a clamp voltage of -31 mV. The arrows indicate the measurement of $I_s$ in these experiments. The beaded appearance of the traces was caused by a minor malfunction of the oscilloscope horizontal position control (the recording oscilloscope was used with the horizontal sweep stopped).
series of clamps of constant amplitude, with the duration varied so as to repolarize the membrane at various times during $I_\alpha$. Immediately after repolarization, the conductance should still be that which was present just before repolarization, but the driving forces on the ions assumed to be responsible for $I_\alpha$ (calcium and sodium) should have increased, whereas those for other ions flowing at the same time as $I_\alpha$ (potassium, and at more positive voltages, chloride) should be abruptly decreased. The envelope enclosing the tail currents should therefore give an indication of the time-course of the conductance change responsible for $I_\alpha$.

We have performed similar experiments to see if the results support our conclusion that the small inward-going deflections on a net outward current give a true indication of the time-course of $I_\alpha$, and also to see if the results are similar to those obtained by others. Fig. 2 illustrates the results of an experiment of this type, for clamps from a holding voltage of $-77 \text{ mV}$ to a clamp...
voltage of $-32$ mV. In the lower part of the figure are records of the current, voltage, and tension for several of the clamps, with the clamp duration (in milliseconds) indicated. Tracings of the total current and the tail currents from the sample records and the other records of the experiment are overlaid at the top of the figure.

The results are very close to those obtained in sheep Purkinje fibers by Vitek and Trautwein (1971) and Reuter (1968), and in cat ventricular muscle by New and Trautwein (1972). The inward tails were largest when the clamp was terminated at the peak of the inward-going deflection, 30–50 ms after the beginning of the clamp, and they were smaller if the clamp was ended either before or after the peak of the deflection. When clamps were terminated very early, tail current actually appeared to stay constant for a short time before beginning to decrease. Similar behavior was observed in cat myocardium by New and Trautwein (1972).

As indicated by Gibbons and Fozzard (1971), tension did not decline until clamp duration was somewhat less than time to peak tension, and as the clamps were made still shorter, the tension declined because of a decrease in the rate of tension development and without the large changes in time to peak tension reported by Morad and Trautwein (1968).

The tracings in the composite drawing of tail behavior were made from the first point at which current was visible on the film, somewhat earlier than it appears in the reproduced examples. Except for the clamps terminated very early, the conductance appeared to be high when the clamp was ended and the end of the capacity current merged with the tail current so that it is difficult to judge the points which should be considered to be the maximum tail currents. There is a reasonably clear break at the end of the capacity current, and if the tails are measured after this point, it appears that there is $I_s$, conductance remaining at 500 ms. An independent estimate of the conductance remaining at the end of 500 ms (in the same preparation), obtained from the data of Fig. 9, is 18% of the maximum reached during the clamp. The data of Fig. 2 would confirm this as a reasonable estimate at this voltage.

The major points of concern are that the inward-going deflection of the current trace does appear to represent the time-course of the conductance change responsible for $I_s$, the tail currents in this rather critical experiment behave like those in working ventricular muscle, and the measurements of $I_s$ probably provide a low estimate of the current. Tension changes as the clamps are made shorter are not related to the $I_s$ flowing before repolarization in any obvious way. We found (Gibbons and Fozzard, 1971) that short clamps were surprisingly effective in causing tension, and if tension depends on $I_s$ the fact that termination of the clamp does not lead to rapid termination of $I_s$ might help explain this earlier observation (see discussion for an alternative explanation).
In Fig. 3 are the results of trains of clamps (1 s⁻¹, 300 ms) from a holding voltage of −68 mV to three different clamp voltages, each within the range where /₈ can be measured. For the train of clamps to −54 mV, there was tension in response to the first clamp of the train, but by the 10th clamp the tension response was not measurable. The slow inward current in response to the 10th clamp is considerably reduced from that obtained during the first clamp.

Increasing the clamp voltage to −43 mV (middle set of records) increased the inward current during the first clamp. Again the current during the 10th clamp is smaller than that obtained during the first clamp, and there is no measurable tension response to the 10th clamp. In the third set of records, there is finally a tension response to the 10th clamp, and the slow inward current has again become smaller in successive clamps during the train.

Beeler and Reuter (1970 b) did not observe changes in the slow inward current (referred to as /₈ in their paper) during repetitive stimulation. Our results probably are not in conflict with theirs, however, since they used a frequency of 0.33 s⁻¹. This frequency allows much more time for /₈ to recover than the 1 s⁻¹ used in these experiments, and considering the rate of recovery of /₈ (see below), we would not expect much change in /₈ at the lower frequency.

When the preparation is clamped to voltages less negative than those in Fig. 3, the changes in /₈ cannot be unequivocally analyzed, as seen in Fig. 4. In the first pair of records for a train of clamps to −22 mV, there is still a negative tension staircase. Outward current is seen during the first clamp, and this outward current declines with position in the train. By the 10th clamp, /₈
can be seen clearly, since the large outward current, which has been identified as being due to chloride movement (Dudel et al., 1967; Reuter, 1968; Fozzard and Hiraoka, 1973), is very much smaller during the 10th clamp.

In the succeeding trains, especially those to \(-1\) mV and to \(+10\) mV, the 10th contraction is larger than the 1st, and between the 1st and the 10th clamps there has been a substantial reduction in the outward current flowing upon depolarization. There is no way of determining what the changes in \(I_s\) may have been, although the experiments of Reuter (1968) and Vitek and Trautwein (1971) indicate that \(I_s\) does flow at these voltages.

Fig. 5 shows more clearly the way in which \(I_s\) changes during a train of voltage clamps. Most of the change in the current is apparent during the second clamp of the train, and as the tracings show, there is very little change in the current after the second clamp. This behavior can be explained by a relatively slow rate of recovery of \(I_s\), after a clamp. If there were any effects due to changes in the reversal potential for \(I_s\), they apparently were small, or else they were reversed during the 700-ms interval between clamps, since there appeared to be little cumulative effect of the repeated depolarization.

Fig. 5 also illustrates another important point. Under our experimental conditions, the threshold voltage at which tension is first seen usually does not coincide with the threshold for \(I_{Na}\). \(I_{Na}\) in this experiment was substantial during clamps to \(-62\) mV and the threshold voltage for \(I_{Na}\) appeared to be \(-67\) mV (not illustrated). The second set of records of Fig. 5 shows tracings from clamps to \(-59\) mV, where \(I_{Na}\) was rather large. There was no tension in

**Figure 4.** Changes in membrane current and tension during trains of voltage clamps. Membrane current (I), membrane voltage (V), and tension (P) for records from four trains to voltages outside the range where \(I_s\) can be measured. Same experiment and arrangement of records as in Fig. 3. (See text.)
response to any clamp at this voltage, and of course there was no tension in response to any of the smaller clamps mentioned above. In addition, the time constant for recovery of $I_{Na}$ is fast enough (Weidmann, 1955) that $I_{Na}$ should not, and did not, change with successive clamps at the frequency used in Fig. 5, yet there are trains in which the clamp voltage is above tension threshold for the 1st clamp and not for the 2nd–10th clamps.

When the holding voltage is made less negative, the threshold for tension shifts (Gibbon and Fozzard, 1971). Depolarization shifts the threshold for $I_{Na}$ as well, so we sometimes find conditions in which the threshold for $I_{Na}$ and that for tension are very close to the same. We do not find, however, any evidence of a causal relation between the two, as suggested by the work of Tritt-hart et al. (1973).

Under the conditions of our experiments, however, tension threshold occurs
at the same voltage where $I_h$ is first detectable. For the first clamp after a long rest, $I_h$ and tension are both present during the first clamp to $-54$ mV, but not present during the first clamp to $-59$ mV (Fig. 5). As indicated in the preceding paper (Gibbons and Fozzard, 1975), the relation between voltage and tension, including tension threshold, may be determined under many different conditions. Perhaps the most physiologic of these is obtained if a steady state is established with a train of clamps, which is then interrupted with a test clamp to different voltages. The behavior of $I_h$ and tension under these conditions is illustrated in Fig. 6, taken from the same experiment illustrated in Fig. 7 of the preceding paper. The 1st, 10th, and 11th (test) clamps are shown for a determination of the voltage-tension relation from a holding potential of $-58$ mV, frequency $1 \text{ s}^{-1}$, clamp duration 300 ms. The first appearance of tension and $I_h$ occurs during the test clamp to $-41$ mV in the second train shown. As the test clamp voltage was increased in the third train, tension and $I_h$ both in-

![Figure 6](image_url)

**Figure 6.** Coincidence of slow inward current and tension thresholds. The records shown were taken from a determination of the voltage-tension relation in which a steady state was established by a train of clamps before the test clamp was imposed (see the inset of Fig. 6, Gibbons and Fozzard, 1975 for the method). The 1st and 10th of the clamps used to establish the steady state before each test are shown; the 11th clamp tests the voltage-tension relation. Clamp voltage for clamps 1-10 was 0 mV, and the voltages of the test clamps are indicated at the right. Slow inward current and tension first appear at $-41$ mV. Holding voltage $-58$ mV, frequency $1 \text{ s}^{-1}$, same experiment and some of the same data as in Fig. 7, Gibbons and Fozzard (1975).
creased. In the last train shown, the voltage during the test clamp (-18 mV) is high enough that the chloride current begins to obscure the behavior of \( I_s \).

During clamps to voltages just barely above threshold for \( I_s \) and tension, we find that the onset of \( I_s \) is considerably slower than it is for stronger depolarizations, and the time to peak tension is usually longer than it is for larger clamps. Both of these effects appear during the clamp to -41 mV in Fig. 6, and in the third set of records in Fig. 5.

In the narrow voltage range between the threshold for \( I_s \) and the voltage at which the chloride current makes measurement of \( I_s \) impossible, we have examined the relation between peak tension and peak slow inward current in several preparations (Fig. 7). The plots are for the first clamp and contraction after a rest, with both \( I_s \) and contraction varying as clamp voltage is increased. To avoid chloride current complications as much as possible, the maximum clamp voltage included in each plot was -40 mV. There clearly is a direct, but probably not a linear, relationship between \( I_s \) and tension. For contractions after a long rest, at least, if there is tension there is also slow inward current (see, for example, Fig. 5), and there appears to be a correlation between the amount of tension and the amount of slow inward current.

The direct relation between \( I_s \) and tension does not hold under all conditions, however. In Fig. 8 are several records from an experiment in which the time-course of recovery of \( I_s \) after a single clamp is examined. The experimental approach is similar to that used by Hodgkin and Huxley (1952) in examining the recovery from inactivation of the sodium current. Both the
initial and the test clamps were 500 ms long, from a holding voltage of -77 mV to a clamp voltage of -32 mV. As the interval between the two clamps was increased, there was clear recovery of $I_s$ before there was any measurable tension in response to the test clamp, and recovery of $I_s$ was complete several seconds before there was complete recovery of the ability to contract.

The data from the complete experiment are plotted as a function of the interval between the two clamps in Fig. 9. Tension and $I_s$ each recovered approximately exponentially. The line fitted to the data for tension was drawn according to the equation $(P)/P_0 = 1 - \exp \left(\frac{-t}{\tau_p}\right)$ with $\tau_p = 3.08$ s. The equation used for the line drawn through the current data was $(I_s)/I_{s0} = 1 - 0.82 \exp \left(\frac{-t}{\tau_I}\right)$, with $\tau_I = 0.67$ s.

The time-course of the recovery of $I_s$ is therefore fast enough to explain why Beeler and Reuter (1970 b) failed to see changes in $I_{Ca}$ during repetitive stimulation at 0.33 s⁻¹. The presence of significant $I_s$ at intervals where tension is not yet measurable, and the continued recovery of the ability to generate tension long after current recovery has peaked indicate that the simple correlation between $I_s$ and tension illustrated in Fig. 7 does not hold under these conditions. Although there still appears to be $I_s$ whenever there is tension, it would seem possible to have $I_s$ without having a (measurable) tension response.

Extrapolation of the current data to zero interval should give the fraction of
Figure 9. Time-courses of slow inward current and tension recovery. Plot of the full data from which the records of Fig. 8 were taken. The peak tension and the peak slow inward current in response to the test clamps, relative to the tension and current during the initial clamp, are plotted as a function of the repolarized interval between the end of the initial clamp and the beginning of the test clamp. The inset illustrates the experimental design and the measurements made. Holding voltage -77 mV, clamp voltage -32 mV.

The $I_s$ system still available at the end of the 500-ms initial clamp. In this experiment, the fraction was 0.18 of the maximum reached during the clamp. There is likely to be considerable error in the measurement of such small currents, if only because of their size. A more confusing problem is that the measurements used to reveal the fraction of the $I_s$ system available at the end of the 500-ms clamp are themselves in error by an amount which depends on the fraction of the system still available at the end of the clamp, since the current at this time was used as the base line from which $I_s$ was measured. The underestimates of the current should be most serious for the smaller currents obtained at short intervals, so it is likely that recovery of $I_s$ occurs somewhat faster than indicated in Fig. 9, and if the exact time-course is important for conclusions more quantitative than we intend to present here, it should be possible to eliminate the errors in this particular determination through an iterative correction procedure. In three experiments of this type, there has been variability in the rate of recovery of $I_s$ and of contractile recovery (see below, and Discussion). The discrepancy between the two rates has been consistent, with $I_s$ recovery faster than recovery of the ability to contract. For the moment it is sufficient to note that there is some reasonably small fraction of
$I_s$, which either does not inactivate or inactivates only very slowly, and the

time-course of $I_s$ recovery is considerably faster than that of tension recovery.

For the curve chosen to describe tension recovery, or "restitution" (Kruta

and Braveny, 1961), the problems are somewhat different. The assumption of

an exponential time-course did not appear to be a bad one, providing that the

curve was chosen to go through the origin. This suggests that tension at very

short intervals was simply less than the noise level of the transducer system. It

should be noted, however, that after clamps to less negative voltages, tension

recovery is much faster than in Fig. 9, and the time-course is decidedly non-

exponential. In particular, there may be an early peak (at an interval of 1–2 s)

followed by a slower phase of recovery (Bautovich et al., 1962; Gibbons and

Fozzard, 1971).

DISCUSSION

The experiments described in this paper support the concept that the slow

inward current is intimately involved in contraction. However, since it is

possible to observe the current under conditions where no tension occurs, the

current probably does not directly cause contraction. Evidence exists for a role

of the current in controlling gradual release from an intracellular calcium

pool and in maintaining the size of the pool. Several aspects of the current and

its relation to contraction deserve further discussion.

Slow Inward Current

The slow inward current observed in these experiments behaves much like

currents described by others in Purkinje preparations (Reuter, 1967, 1968;

Vitek and Trautwein, 1971), working mammalian ventricle (Mascher and

Peper, 1969; Beeler and Reuter, 1970 a), and frog myocardium (Rougier

et al., 1969). In some experiments and at some voltages, $I_s$ was seen as a net

inward current early in the clamp, as in Figs. 1, 3, and 5, while in others it

appeared as an inward-going deflection of a net outward current as in Figs. 2

and 8. The behavior of the current did not seem to depend on whether net

current was inward or outward.

While most of the slow inward current was phasic, examination of the tail

currents occurring when the preparation was repolarized at various times
during $I_s$ suggests that under some conditions the $I_s$ system is not completely

inactivated during long clamp steps. This conclusion is supported by data on

the recovery of $I_s$, but we recognize potential errors in the measurements

which leave us reluctant to say how large that residual fraction may be. In

any case, we would expect it to be a function of voltage, so the fact that such a

fraction exists is perhaps more important at the moment than the exact

amount remaining under a particular set of conditions.

The $I_s$ system recovers slowly enough after a depolarization to explain the
changes in $I_s$, seen here during a train of clamps, yet rapidly enough to explain the fact that Beeler and Reuter (1970 b) and New and Trautwein (1972) saw no changes in the slow inward current during repeated clamps at a lower frequency.

The data of Beeler and Reuter (1970 a) and Bassingthwaighte and Reuter (1972) indicate that the equilibrium voltage for calcium changes as the calcium current flows into the muscle. Bassingthwaighte and Reuter (1972) interpret their results as showing that calcium flows into a restricted space, which has a volume on the order of 1% of the muscle volume. Whether or not the calcium flows into a restricted space is important in understanding cardiac excitation-contraction coupling and for any determination of the kinetics of the $I_s$ system. We could not determine directly if there were changes in the equilibrium voltage for $I_s$ because of the large outward currents flowing at positive voltages. Unless any such accumulation effects are very rapidly reversed, however, we might have expected to see changes in $I_s$ with position in a train, over and above those changes attributable to the relatively slow recovery of $I_s$ after a clamp. Definite proof of calcium accumulation in a limited space could require monitoring of the electrical potential in this restricted space, and the necessary experiments are not technically feasible.

Relation of $I_s$ to Contraction

Since an increase in the concentration of calcium in the muscle is apparently the final event coupling excitation and contraction, a voltage-dependent inward movement of calcium might affect contraction. Several possible roles for the current can be suggested. The current might represent a major source of calcium for direct activation of contraction. By “direct,” we mean that most of the calcium which activates a contraction would come from the immediately preceding slow inward current. If this were true, there would have to be certain time relationships between the current and contraction, contraction should be proportional to the amount of current, and it should not be possible to see current without contraction. Alternatively, the current might regulate the amount of calcium in an intracellular pool, which in turn governs contraction. In this case, there might be a correlation between steady-state contraction and $I_s$. Finally, current may influence release of calcium from an intracellular pool, either as a trigger or as a regulator. If it acts as a trigger, the contraction should not be proportional to current. Some evidence for each role can be mentioned.

Unless calcium entry via the slow inward current occurs into a restricted space and is somehow prevented from helping to activate the myofilaments during a particular depolarization, a direct role in contraction seems at least to be possible. The current preceded contraction. When the first contractions after a rest were examined, the voltage at which tension was first seen was the
same as the threshold for $I_s$. For clamps just barely above threshold for $I_s$ and tension, the onset of $I_s$ was slow, and time to peak tension was prolonged. Under a fairly wide range of conditions, contraction was associated with $I_s$, in the sense that if there was contraction, there also was slow inward current. Although a direct contribution of calcium entering via $I_s$ does appear possible, it seems unlikely that $I_s$ could be the major source of activating calcium. If it were, there should be a direct relation between $I_s$ and tension under all conditions. For the first contraction after a rest, there was a correlation between $I_s$ and peak tension in the voltage range where we could make meaningful measurements. The relation was not linear, but since the base line for current measurement probably did not represent a true zero for $I_s$, the current magnitudes were likely in error. However, under the conditions used to examine recovery of $I_s$ and tension, the two did not recover together, or even along similar time-courses. Slow inward current recovered more rapidly than tension, and $I_s$ recovery was complete before the muscle had fully recovered the ability to contract. Similarly, tension threshold seemed to shift to more positive voltages in the course of a train of clamps, so that there could be slow inward current without any measurable steady-state contractions. Even recognizing the variability of the rate of contractile recovery, which might cause relations between $I_s$ and tension different from those in Fig. 9 if different conditions were used, we must conclude that most of the calcium which activates contraction does not come directly from $I_s$.

The alternative is that the immediate source of the calcium which activates contraction is an internal pool. The cumulative effects of repeated depolarization described in the preceding paper (Gibbons and Fozzard, 1975) convince us that there is a variable internal pool of calcium which has a substantial effect on contraction, and $I_s$ is the most likely source of calcium to replenish or augment that pool. Others (Wood et al., 1969; Morad and Trautwein, 1968; Beeler and Reuter, 1970b; New and Trautwein, 1972; Tritthart et al., 1973) have reached a similar conclusion. In addition, there are other observations reported here which are consistent with this view. During trains, the most dramatic change in $I_s$ occurred in the 2nd clamp of the train; there was little, if any, change between the 2nd and the 10th depolarizations. Yet the tension continued to change for several more beats before reaching a steady state.

While our evidence and that of others favors the conclusion that there is a variable internal pool there is not a simple and direct relation between $I_s$ and steady-state tension, as one might expect. While the threshold for $I_s$ and tension seemed to be identical after a rest, we have already noted that in the steady state there could be $I_s$ without measurable tension. Furthermore, although we could not measure $I_s$ during clamps to positive voltages, we would expect $I_s$ to decrease during very strong depolarizing clamps. In the preceding paper (Gibbons and Fozzard, 1975), we did observe that the first contraction
after a rest began to decrease as the clamp voltage was increased to inside positive potentials. At these same voltages, however, the steady-state contractions did not decrease, and in fact a positive staircase was seen at the same voltages which decreased the peak tension of the first contraction after a rest.

It is not necessary to modify the idea that $I_s$ adds calcium to an intracellular pool in order to explain these results, however. If the $I_s$ system does not inactivate completely during the 300-ms clamps used, there would be substantial calcium entry at the end of each clamp in a train of clamps to inside positive voltages, because of the sudden increase in the driving force on calcium at the end of each depolarization. This might well compensate for any decrease in $I_s$ during the clamps themselves. One of the problems in this and in other arguments put forth concerning the possible effects of the slow inward current on the size of intracellular stores is that one also needs to consider how calcium may leave the cell.

Having concluded that there is a variable internal pool of calcium, the problem is to explain the observations which showed a correlation between peak $I_s$ and peak tension for rest contractions, the identical thresholds for $I_s$ and tension under these conditions, and the prolonged time to peak $I_s$ and time to peak tension for barely suprathreshold clamps. This intimate relation between $I_s$ and tension was present after a long rest, when the internal pool should be constant. When we would expect the size of the pool to be changing, for example during recovery, the relationships broke down. We think the simplest way to reconcile these observations is to assume that the release of calcium from the intracellular pool is linked to $I_s$, so that calcium entry causes the release of a proportional amount of calcium from the pool. Under those circumstances where the pool is depleted, as it may be after repeated low voltage clamps or at the beginning of recovery, it would then be possible to have $I_s$ without having a tension response. It would not be possible, however, to have a tension response to a voltage clamp unless it was preceded by $I_s$.

The time relationships, the coincidence of $I_s$ and tension thresholds, and the correlation between $I_s$ and tension are consistent with the involvement of $I_s$ in release from an intracellular pool. Such a role for $I_s$ also makes it much easier to see how very brief clamps could activate contractions which were much smaller than, but had the same time-course as, those activated by much longer clamps.

There is ample evidence that an increase in the intracellular calcium concentration can trigger the release of additional calcium from the sarcoplasmic reticulum, especially if it is loaded with calcium (Endo et al., 1970; Ford and Podolsky, 1972), and the mechanism has recently been demonstrated in mechanically disrupted heart cells (Kerrick and Best, 1974). In the Purkinje fiber, however, the calcium entry appears to cause a proportional release of the calcium from the intracellular store, rather than a regenerative release.
We conclude, then, that there probably is a variable intracellular pool of calcium, and that the best explanation of our data presently is that calcium entering by way of the slow inward current has at least two effects. It causes the release of additional calcium from this pool, and it can replenish or increase the size of the calcium pool. A direct contribution of calcium entry to contraction is possible, but acceptance of the possibility that $I_s$ regulates release from internal stores makes it unnecessary to assume that the direct contribution is large.

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REFERENCES

BASINGTHWAITE, J. B., and H. REUTER. 1972. Calcium movements and excitation-contraction coupling in cardiac cells. In Electrical Phenomena in the Heart. W. C. DeMello, editor. Academic Press, Inc., New York. 353.

BAUTOVICH, G., D. B. GIBB, and E. A. JOHNSON. 1962. The force of contraction of the rabbit papillary muscle preparation as a function of the frequency and pattern of stimulation. Aust. J. Exp. Biol. Med. Sci. 40:455.

BEELER, G. W., Jr., and H. REUTER. 1970a. Membrane calcium current in ventricular myocardial fibres. J. Physiol. (Lond.). 207:191.

BEELER, G. W., Jr., and H. REUTER. 1970b. The relation between membrane potential membrane currents, and activation of contraction in ventricular myocardial fibres. J. Physiol. (Lond.). 207:211.

DUDEL, J., K. PEPER, R. RÜDEL, and W. TRAUTWEIN. 1967. The dynamic chloride component of membrane current in Purkinje fibers. Pflugers Arch. Eur. J. Physiol. 295:197.

ENDO, M., M. TANAKA, and Y. OGAWA. 1970. Calcium induced release of calcium from the sarcoplasmic reticulum of skinned skeletal muscle fibres. Nature (Lond.). 228:34.

FORD, L. E., and R. J. PODOLSKY. 1972. Intracellular calcium movements in skinned muscle fibres. J. Physiol. (Lond.). 223:21.

FOZZARD, H. A., and D. C. HELLAM. 1968. Relationship between membrane voltage and tension in voltage-clamped cardiac Purkinje fibers. Nature (Lond.). 218:588.

FOZZARD, H. A., and M. HRADKA. 1973. The positive dynamic current and its inactivation properties in cardiac Purkinje fibres. J. Physiol. (Lond.). 234:569.

GIBBONS, W. R., and H. A. FOZZARD. 1971. Voltage dependence and time dependence of contraction in sheep cardiac Purkinje fibers. Circ. Res. 28:446.

GIBBONS, W. R., and H. A. FOZZARD. 1975. Relationships between voltage and tension in sheep cardiac Purkinje fibers. J. Gen. Physiol. 65:245.

HODGKIN, A. L., and A. F. HUXLEY. 1952. The dual effect of membrane potential on sodium conductance in the giant axon of Loligo. J. Physiol. (Lond.). 116:497.

KERRICK, W. G. L., and P. M. BEST. 1974. Calcium ion release in mechanically disrupted heart cells. Science (Wash. D. C.). 185:435.

KRUTA, V., and P. BRAVENY. 1961. Restitution de la contractilité du myocarde entre les contractions et les phénomènes de potentiation. Arch. Int. Physiol. Biochim. 69:645.

MASCHER, D., and K. PEPER. 1969. Two components of inward current in myocardial muscle fibers. Pflugers Arch. Eur. J. Physiol. 307:190.

MORAD, M., and W. TRAUTWEIN. 1968. The effect of the duration of the action potential on contraction in the mammalian heart muscle. Pflugers Arch. Eur. J. Physiol. 299:56.
New, W., and W. Trautwein. 1972. The ionic nature of slow inward current and its relation to contraction. Pfluegers Arch. Eur. J. Physiol. 334:24.
Reuter, H. 1967. The dependence of slow inward current in Purkinje fibres on the extracellular calcium concentration. J. Physiol. (Lond.). 192:479.
Reuter, H. 1968. Slow inactivation of currents in cardiac Purkinje fibres. J. Physiol. (Lond.). 197:233.
Reuter, H. 1973. Divalent cations as charge carriers in excitable membranes. Prog. Biophys. Mol. Biol. 26:1.
Rouquier, O., G. Vassort, D. Garnier, Y. M. Gargouil, and E. Coraboruf. 1969. Existence and role of a slow inward current during the frog atrial action potential. Pfluegers Arch. Eur. J. Physiol. 308:91.
Trautwein, W. 1973. Membrane currents in cardiac muscle fibres. Physiol. Rev. 53:793.
Tritthart, H., R. Kaufmann, H.-P. Volkmer, R. Bayer, and H. Krause. 1973. Ca-movement controlling myocardial contractility. I. Pfluegers Arch. Eur. J. Physiol. 338:207.
Vitek, M., and W. Trautwein. 1971. Slow inward current and action potential in cardiac Purkinje fibres. Pfluegers Arch. Eur. J. Physiol. 323:204.
Weidmann, S. 1955. The effect of the cardiac membrane potential on the rapid availability of the sodium carrying system. J. Physiol. (Lond.) 127:213.
Wood, E. H., R. L. Heppner, and S. Weidmann. 1969. Inotropic effects of electric currents. Circ. Res. 24:409.