

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 Bassingthwaite 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 (Bassingthwaite 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

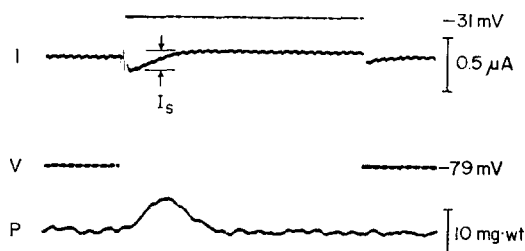


FIGURE 1. General characteristics of the slow inward current and its relation to contraction. Membrane voltage (V), membrane current (I), and tension (P) are shown for a 500-ms clamp from a holding voltage of -79 mV to a clamp voltage of -31 mV. The arrows indicate the measurement of I_s in these experiments. 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_s . 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_s (calcium and sodium) should have increased, whereas those for other ions flowing at the same time as I_s (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_s .

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_s , 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 mV to a clamp

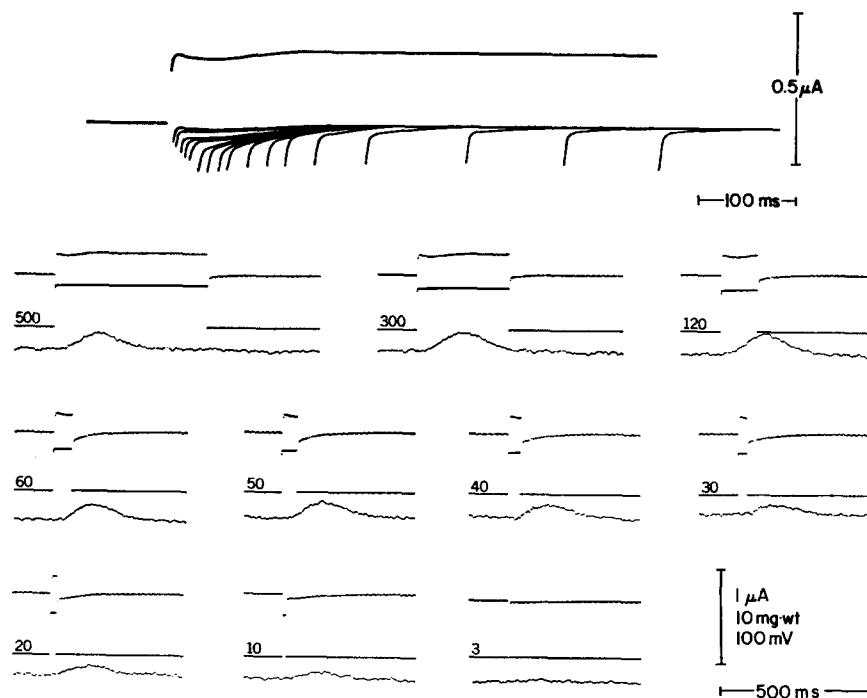


FIGURE 2. Time-course of the slow inward current conductance as estimated by inward current tails. Single voltage clamps, after a rest of at least 90 s, were given from a holding voltage of -77 mV to a clamp voltage of -32 mV. The tails of inward current occurring upon repolarization were traced for clamp durations of 500–2 ms; these are overlaid at the top of the figure. Below the tracings are some of the records from which the tracings were made, with the clamp duration (in milliseconds) indicated for each record. From the top down in each record, the oscilloscope traces are membrane current, membrane voltage, and tension. (See text for discussion.)

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 I_s 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,

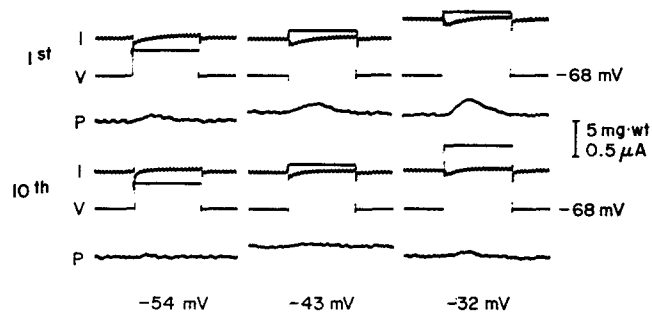


FIGURE 3. Changes in the membrane current and tension during trains of voltage clamps. In this and in subsequent figures, a train is defined as a series of voltage clamps beginning after a rest of at least 90 s. Membrane current (I), membrane voltage (V), and tension (P) records are shown for the 1st (upper) and 10th records (lower) during three trains. Clamp voltages for each train are within the range where I_s can be measured; the voltages used for the three trains are indicated below the records. The holding voltage was -68 mV , and the frequency was 1 s^{-1} for each train.

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 I_{Ca} 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 I_s to recover than the 1 s^{-1} used in these experiments, and considering the rate of recovery of I_s (see below), we would not expect much change in I_s at the lower frequency.

When the preparation is clamped to voltages less negative than those in Fig. 3, the changes in I_s 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, I_s

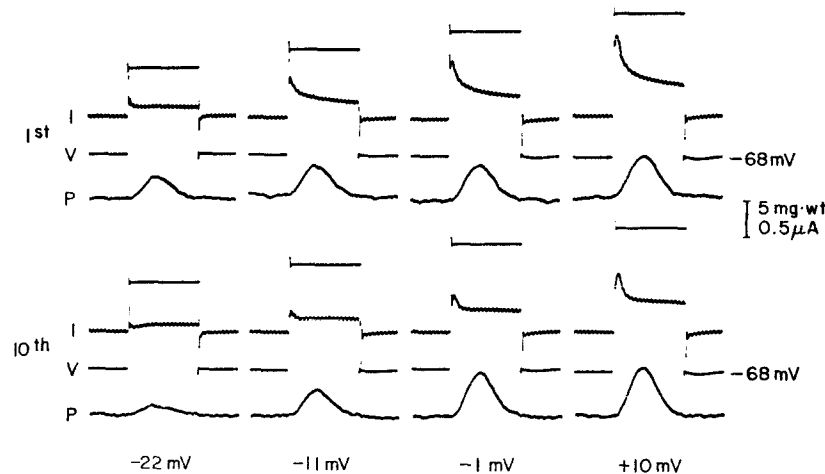


FIGURE 4. Changes in membrane current and tension during trains of voltage clamps. Membrane current (I), membrane voltage (V), and tension (P) for records from four trains to voltages outside the range where I_s can be measured. Same experiment and arrangement of records as in Fig. 3. (See text.)

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

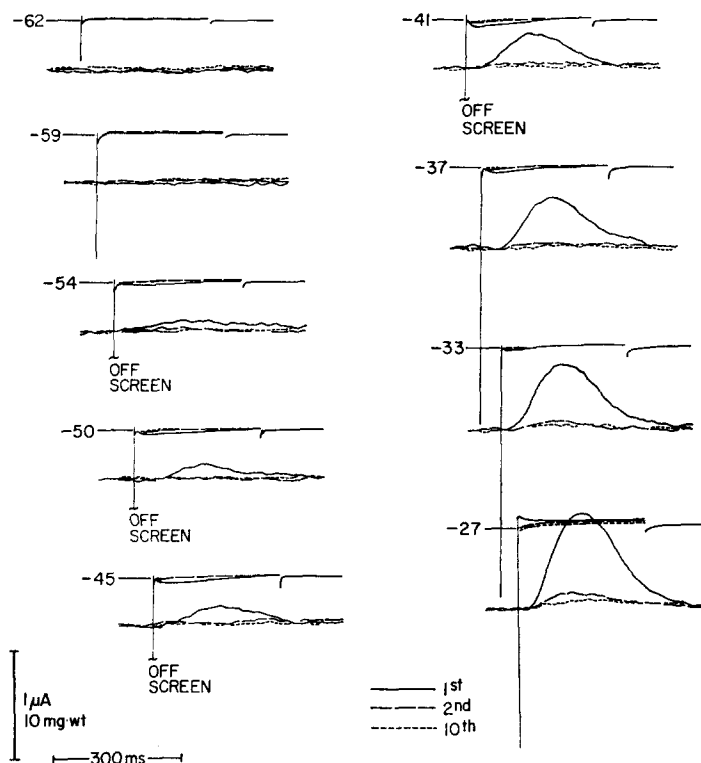


FIGURE 5. Membrane current and contraction during voltage clamp trains. The membrane current (upper trace in each record) and tension (lower and noisier trace in each record) were traced and overlaid for the 1st, 2nd, and 10th clamps of nine trains to the voltages indicated. The record from the 1st clamp is shown as a solid line, the 2nd as a dashed line, and the 10th as a dotted line. At the gain used to see the slow inward current, the peak sodium current was off screen on some records. Holding voltage -75 mV, clamp duration 300 ms, frequency 1 s^{-1} .

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 Tritthart et al. (1973).

Under the conditions of our experiments, however, tension threshold occurs

at the same voltage where I_s is first detectable. For the first clamp after a long rest, I_s 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_s 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 s^{-1} , clamp duration 300 ms. The first appearance of tension and I_s 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_s both in-

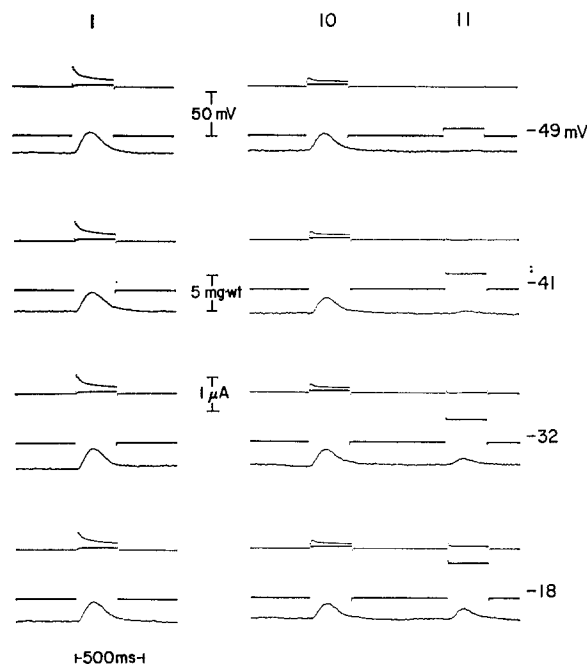


FIGURE 6. Coincidence of slow inward current and tension thresholds. The records shown were taken from a determination of the voltage-tension relation in which a steady state was established by a train of clamps before the test clamp was imposed (see the inset of Fig. 6, Gibbons and Fozzard, 1975 for 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 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

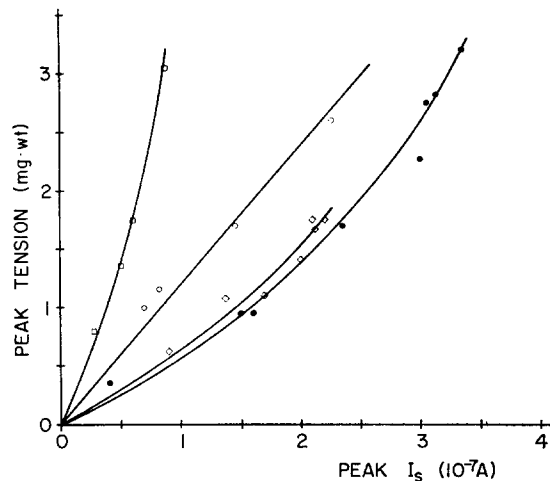


FIGURE 7. Relation between slow inward current and tension near threshold. Peak tension is plotted as a function of peak slow inward current for four preparations. Data were taken from the responses to the first voltage clamps after 90 s or longer rests, for clamp voltages just above the threshold for I_s and tension. The curves were drawn by eye.

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

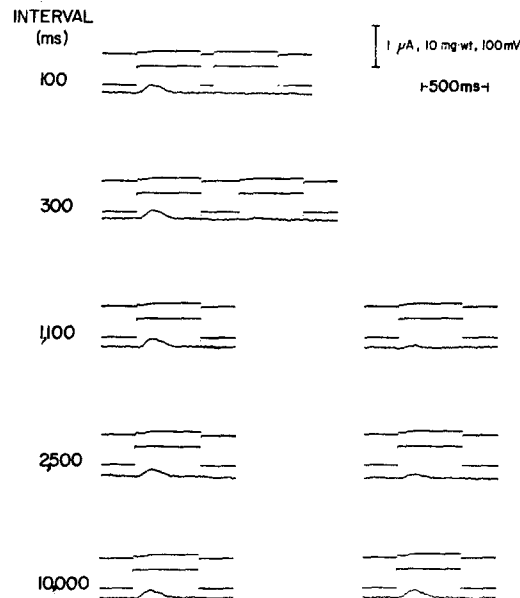


FIGURE 8. Recovery of slow inward current and tension. Recovery was examined by varying the repolarized interval between two identical 500-ms voltage clamps. A rest of at least 90 s preceded the first of the two clamps. The interval (in milliseconds) is shown at the left of each pair of voltage clamps. Holding voltage -77 mV, clamp voltage -32 mV, same preparation as in Fig. 2.

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 $(P)_t/(P)_o = 1 - \exp(-t/\tau_p)$ with $\tau_p = 3.08$ s. The equation used for the line drawn through the current data was $(I_s)_t/(I_s)_o = 1 - 0.82 \exp(-t/\tau_I)$, 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

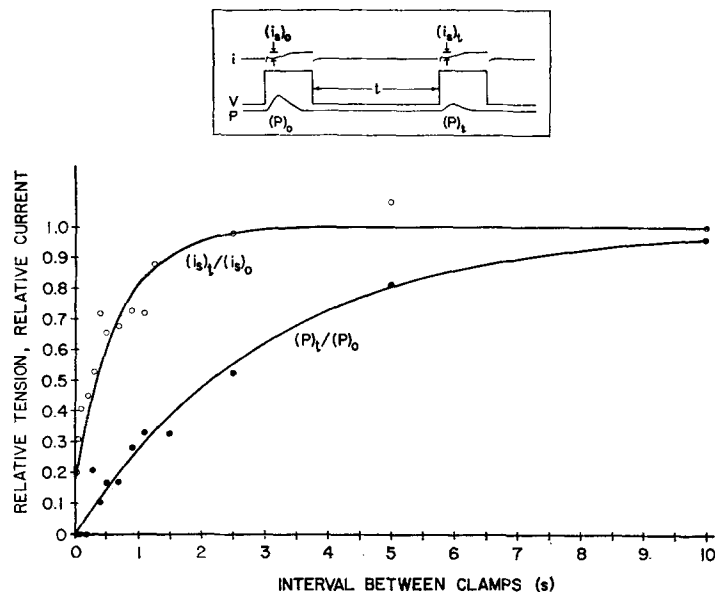


FIGURE 9. Time-courses of slow inward current and tension recovery. Plot of the full data from which the records of Fig. 8 were taken. The peak tension and the peak slow inward current in response to the test clamps, relative to the tension and current during the initial clamp, are plotted as a function 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 Bassingthwaighe and Reuter (1972) indicate that the equilibrium voltage for calcium changes as the calcium current flows into the muscle. Bassingthwaighe 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, 1970 *b*; 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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