Restitution of the action potential in cat papillary muscle

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Bass, Berl G. Restoration of the action potential in cat papillary muscle. Am. J. Physiol. 228(6): 1717–1724. 1975.—Membrane recovery (electrical restitution) following an action potential (AP) was studied in isolated, electrically driven cat papillary muscle by interpolation of test extrasystoles at various delays in the interval between two APs (recorded with glass microelectrodes). Curves describing the time course of restitution were derived by plotting extrasystolic AP area versus delay preceding the extrasystole. Electrical restitution begins with slow dV/dt, short plateau, and small AP area, then proceeds through a phase of large AP area with long positive plateau (phase A of electrical restitution) before control AP characteristics recur. APs during phase A resemble low-Ca++ APs. AP characteristics reciprocal to those during phase A persist for three to four postextrasystolic APs and resemble high-Ca++ APs. Phase A and postextrasystolic AP changes are exaggerated in low Ca++ and minimal in high Ca++. These observations suggest an inverse relationship between membrane Ca++ content and AP area and the existence of a negative feedback loop by which AP configuration is controlled. It is proposed that phase A, which occurs approximately concurrently with phase I of mechanical restitution (1), may be due to a transient post-AP decrease in membrane-bound Ca++ occurring concurrently with the transient elevation of intracellular free Ca++ discussed previously (1). The observations suggest an inverse relationship between membrane Ca++ content and AP area and plateau duration, and the existence of a negative feedback loop, based on this relationship, by which APs control their own configuration.

METHODS

Cats of 1.5–3.9 kg were anesthetized intraperitoneally with pentobarbital sodium (40 mg/kg). Hearts were excised and right ventricular papillary muscles were removed and prepared for study as described previously (1). All muscles were mounted horizontally in a chamber lined with beeswax and maintained at constant temperature of 23–25°C by a circulating-water bath. This range of temperatures, although low for studies of transmembrane potential, was chosen because it lay within the range of temperatures of electrical restitution in the rabbit had been reported (9), and another, in the dog (19), appeared after the current investigation was started. No studies of this type in cat ventricular muscle were available. Electrical restitution (E-R) was therefore investigated in cat papillary muscle by expanding the previous study of the restitution of contractility to include simultaneous transmembrane AP recordings.

The results of this investigation of the normal time course of electrical restitution are described in the present paper. They indicate that in cat papillary muscle the membrane, like the contractile apparatus, goes through a specific period of recovery after an AP. These post-AP changes are exaggerated in low Ca++ and are less apparent in high Ca++. It is suggested that they may be a manifestation of a transient post-AP decrease in membrane-bound Ca++ occurring concurrently with the transient elevation of intracellular free Ca++ discussed previously (1). The observations suggest an inverse relationship between membrane Ca++ content and AP area and plateau duration, and the existence of a negative feedback loop, based on this relationship, by which APs control their own configuration.
transmembrane potentials were measured by means of Pyrex glass microelectrodes with tip diameters of approximately 0.5 \( \mu \)m and resistances of 10\,000 M\( \Omega \). The microelectrodes were filled with 2.8 M KCl by boiling under reduced pressure. They were positioned with a multidirectional micromanipulator and inserted under direct binocular microscopic control as close to the tip of the papillary muscle as possible to avoid impalement of Purkinje or juncional cells (18). However, stable impalements in this region were frequently difficult to achieve because of the greater degree of movement encountered at the muscle tip. Most successful experiments were therefore from cells in the middle third of the muscle. Floating microelectrodes, as described by Woodbury and Brady (28), were used in a few experiments, but did not seem to offer any advantages over standard glass microelectrodes with long flexible tips.

In most instances, a 0.005-inch tungsten wire was sealed into the KCl-filled lumen of the microelectrode and then connected directly either to the input of a DC amplifier with input-capacity neutralization (Bioelectric Instruments) or to a field-effect transistor used as a source follower. The latter, with an input capacity of less than 5 pF, was considered adequate when phase 0 of the action potential (12) was not of particular concern. A W-P Instruments RC-1 indifferent electrode was placed directly in the chamber. In some experiments the microelectrode was held in a Bioelectric Instruments MH 1 microelectrode holder and connected to the source follower or amplifier via a Ag-AgCl wire. The indifferent electrode in such experiments was a Ag-AgCl wire in 2.8 M KCl connected to the chamber by a KCl-agar bridge. The DC amplifier or source-follower output was fed through an operational amplifier into an Ampex SP-300 tape recorder. Tension was measured simultaneously and recorded on a second tape channel, and time marks, generated by a Tektronix 161 pulse generator, were measured and recorded on a third.

As soon as acceptable AP's were recorded (control AP's with resting potentials of less than \(-80\) mV were rejected), a control E-R curve was begun. E-R curves were derived by the interpolation of extrasystolic stimuli in the same way as described for the derivation of M-R curves (1). After completion of the control E-R curve, the chamber was rapidly emptied, rinsed, refilled with either a low- (0.625 mM) or high- (7.5 mM) Ca\(^{++}\) solution, the muscle was equilibrated for about 35 min (variable), and a repeat E-R curve was obtained. As far as possible, attempts were made to complete the set of E-R curves with the microelectrode in the same cell. This, of course, was exceedingly difficult. If the microelectrode tip slipped out of the cell, it was immediately reinserted into (hopefully) the same or an adjacent cell. If this could be accomplished within a few minutes, the curve was continued; if not, the curve was restarted after another cell was successfully impaled. Zero potential was always recorded before any impalement and at the end of an experiment. Because it was desirable as far as possible to complete E-R curves from a single impalement, electrodes were deliberately not withdrawn to record zero potential during a study. Since some baseline drift was unavoidable, records therefore do not show a zero potential. Because of the above difficulties, many experiments were considered incomplete or inadequate and were excluded from this investigation. The total number of experiments in this study is thus relatively small.

The tape-recorded records of AP's (and tension) were played into a Grass polygraph and recorded at a paper speed of 100 mm/s. Selected records from each experiment were also played into a Tektronix 564 B storage oscilloscope and photographed. AP's were then analyzed for amplitude, plateau voltage and duration, total duration (at 90\% repolarization), area under the AP (integrated by planimetry), and, at times, \( dV/dt \). The area of early, partially fused extrasystolic AP's was determined by subtracting from the area of the fused (control + extrasystolic) AP the area of the control AP immediately preceding it. AP area was chosen as the index of variability for the E-R curves in this study. E-R curves were derived by plotting the area of the extrasystolic AP against the time in the cycle preceding the extrasystolic stimulus. Changes in other parameters of the interpolated AP's are also discussed below.

**RESULTS**

Normal electrical restitution. Electrical restitution was studied successfully in 24 cat papillary muscles. Twenty-two adequate control E-R curves were derived. Figure 1 illustrates the typical course of electrical restitution under control conditions. Extrasystolic stimuli interpolated very early in the interval elicited AP's that began before repolarization of the control AP was complete (Fig. 1, AP's a, b, and c). Some degree of conduction block occasionally was observed at this time, and in such instances these AP's were not included in the E-R curves. The partially fused AP's were usually subnormal in area, confirming previous observations that AP's occurring during terminal repolarization are "graded" (4, 15, 18, 26). In addition to a smaller resting potential, partially fused AP's characteristically had a small and abbreviated period of initial rapid repolarization (phase I), a short plateau (phase 2) that remained at a more negative potential than normal, and a slower rate of depolarization (\( dV/dt \)). Figure 2, taken from a different experiment, shows that the \( dV/dt \) of partially fused extrasystolic AP's was inversely related to their degree of prematurity.

AP's interpolated slightly later in the interval were normal in area and total duration (Fig. 1, APd), but \( dV/dt \) remained slower than normal, phase I was usually absent, and the
FIG. 1. Restitution of action potential. Upper half of figure shows selected action potentials (APs) interpolated at intervals from 450 to 960 ms (a–f) after their preceding control APs (superimposed on left). Drive interval = 1,470 ms, temperature = 23.0°C, time marks every 50 ms. Stimulus artifacts for each extrasystolic action potential (APES) can be seen below resting-potential level. Part of superimposed first postextrasystolic APs (at end of 1,470-ms interval) can be seen on right. Upper and lower halves of figure are plotted on same time scale. Lower half of figure shows electrical restitution curve for this muscle, derived by plotting APES area, determined by planimetry, against cycle delay preceding APES. Areas of extrasystolic APs a–f are indicated on curve. During course of electrical restitution (E-R), AP area passes through a greater than normal phase (phase A).

plateau tended to remain at zero potential level for considerably longer than normal.

Of special interest was the course of electrical restitution still later in the interval. AP areas became progressively greater with greater delay after the control AP, reached a peak (Fig. 1, AP), and then, with still greater delays, returned toward normal (AP). The area of AP, in Fig. 1 was 45% greater than control. AP area did not return to control level in that experiment before 65% of the interval between AP's (960 ms). A similar phase of increased AP area during the course of electrical restitution (which will be referred to as phase A) was observed in 90% of the 22 control E-R curves. Maximal AP area during phase A was variable and ranged from 11 to 70% greater than control. Phase A duration also was variable and ranged from 18 to 71% of the interval.

In addition to increased AP area, three other characteristics typical of AP's during phase A under control conditions are shown in Fig. 3. They are: 1) phase I (initial rapid repolarization) usually was absent, or was small and brief, 2) the plateau occurred at a more positive potential than normal, and was more prolonged, and 3) the slope of phase 3 repolarization was steeper than normal. AP duration (at 90% repolarization), although slightly prolonged in 65% of the control experiments, was variable. The consistent increase in AP area was due primarily to the more positive and prolonged plateau. The slow dV/dt generally reached normal by the peak of phase A.

Effect of an interpolated extrasystole on postextrasystolic AP's. The extrasystolic AP (APES), with its large area, small or absent phase I, more positive and longer plateau and rapid repolarization, was followed by a first postextrasystolic AP (APES) with almost exactly reciprocal characteristics. These can also be seen in Fig. 3. The APES was characterized by: 1) a small area; 2) a prominent phase I; 3) an extremely brief plateau, which was sometimes not distinguishable as a true plateau and which occurred at a more negative voltage; and 4) a shallow phase 3 repolarization slope. AP duration at 90% repolarization was highly variable. The greatest changes were again in phase 2.

The changes induced in postextrasystolic AP's by the interpolation of a single extrasystole persisted briefly (Fig. 4d) and were variable in type. They were always most marked in AP and then gradually disappeared, usually by APES. The variability of the changes is illustrated in Figs. 4B and 5.

FIG. 2. Rate of depolarization (dV/dt) of early partially fused extrasystolic AP's. Oscilloscope was triggered by each control and each extrasystolic AP. Extrasystoles were interpolated with delays of 405, 430, 460, and 515 ms after the beginning of control AP. Four control AP's are superimposed. Greater degree of partial fusion (smaller resting potential) of earlier extrasystolic AP's is associated with slower dV/dt and less total depolarization.

FIG. 3. Characteristics of a single interpolated extrasystolic AP (APES) and subsequent postextrasystolic AP (APES). Record is from same experiment as Fig. 1. Control AP to left was one of a regular sequence; AP to right was another in that sequence, except that an extra AP (APES) was interpolated between the two (without breaking rhythm). Delay before APES was 615 ms. Absent phase I prolonged positive phase 2, rapid phase 3, and large area of APES are in contrast to opposite characteristics of APES.
The relationship between the areas of the extrasystolic and PES-1 AP's is shown in more detail by the single experiment in Fig. 7. The inverse relationship held in a general way only when the APES was greater than normal (after 500 ms in this experiment) and was linear only after the peak of phase A (after 650 ms).

Effects of [Ca++] on phase I of mechanical restitution is related in part to an elevation of the intracellular "free" [Ca++], and if, as is generally believed, most of this Ca++...
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originates from intracellular stores, then a transient lowering of the Ca++ content of these stores, which presumably include the cell membrane, might occur concomitantly with phase I. The question therefore arose whether phase A of electrical restitution might be the reverse of phase I of mechanical restitution and be in some way related to “lowered” membrane Ca++ levels, i.e., whether early electrical and mechanical restitution (phases A and I) might not both be manifestations, at least in part, of the same cellular change. The effects of [Ca++] on phase A were therefore investigated.

EFFECTS OF LOW Ca++. Low Ca++ (0.625 mM) was studied in five muscles. Control APs in low Ca++ had large areas secondary to a prolonged plateau and a longer duration, as previously observed (14). They were similar in appearance to extrasystolic APs in normal Ca++, as shown in Fig. 8B. The findings illustrated in Fig. 8 were typical of all five muscles and indicate that all the usual characteristics of extrasystolic and PES-1 APs found under control conditions were present in low Ca++, but were exaggerated. The inverse relationship between the areas of the extrasystolic and PES-1 APs was also more marked. This exaggeration of the normal by low Ca++ was evident in the low-Ca++ E-R curve (Fig. 9), in which the degree and duration of phase A were increased.

EFFECTS OF HIGH Ca++. High Ca++ (7.5 mM) was studied in five muscles. Control APs in high Ca++ had small areas secondary to a shortened plateau, as previously observed (14). They were similar in appearance to PES-1 APs in normal Ca++ (Fig. 10). The changes normally observed in extrasystolic and PES-1 APs, and the reciprocal relationship between their areas, were all present in high Ca++ but were minimal. The degree and duration of phase A of electrical restitution were usually decreased in high Ca++ (Fig. 10), but the change from the control E-R curve was not marked.

DISCUSSION

Shortly after the onset of a contraction of cardiac muscle, the process of recovery of contractility (mechanical restitution) begins. The time course of mechanical restitution was described in the preceding paper (1). The experiments

FIG. 9. Electrical restitution in low Ca++. Solid circles are control E-R curve, open circles E-R curve in low Ca++. Ringer data point in each curve refers to APES shown in Fig. 8. Phase A of electrical restitution is exaggerated in low Ca++.

FIG. 10. Effects of 7.5 mM Ca++ on extrasystolic and postextrasystolic APs. Drive interval = 2,880 ms, temperature = 25.0°C. Arrangement of records as in Fig. 8. In high Ca++ (lower records), control AP plateau is shortened, extrasystolic AP changes, although still present, are not as pronounced, and postextrasystolic AP changes are minimal. First postextrasystolic AP under control conditions resembles control AP in high Ca++. E-R curves from these records are shown in Fig. 11.
described in the present paper show that the transmembrane AP also goes through a specific time course of recovery, electrical restitution. In addition, just as a period of altered contractility (postextrasystolic potentiation) persists for six to eight contractions after an extrasystole, a period of altered AP's is also present for three to four postextrasystolic AP's.

Alterations of AP shape associated with an extrasystole, similar to those reported in this paper for cat ventricular muscle, have also been observed in ventricular and atrial muscle of other species. Prolongation of phase 2 of interpolated extrasystolic AP's has been reported in ventricular muscle of dogs (11, 13, 19, 20) and rabbits (8) and in atria of dogs (10) and guinea pigs (6). The subsequent shortening of phase 2 and prolongation ("tailing off") of phase 3 in the first postextrasystolic AP has also been observed in dog (11, 13), rabbit (9) and guinea pig (7) ventricular muscle, and in dog atria (10).

Bravenč and Šumbera (7) have shown that an alteration in the shape of sheep ventricular AP's can be induced even in the absence of any change in the basic regular drive rhythm. If a single regularly driven AP is artificially prolonged by application of a depolarizing direct current (through a sucrose gap), the next (normally driven) AP has a short phase 2 and more gradual phase 3 and is followed by AP's with similar characteristics that gradually disappear. Wood, Heppner, and Weidmann (27) failed to find significant AP changes after termination of depolarizing direct-current pulses in a similar preparation, but this difference may be due to differences in the intensity, duration, and timing of the pulses.

Possible mechanisms underlying electrical restitution and postextrasystolic AP changes. The observations reported in this paper suggest that Ca++ ions may play a role in whatever mechanism(s) underlie electrical restitution and postextrasystolic AP changes. The characteristics of extrasystolic AP's during phase 4 are remarkably similar to those of control AP's in low Ca++. The subsequent three to four postextrasystolic AP's then have the characteristics of high Ca++ AP's. The high-Ca++-like characteristics are most marked in the first AP after the extrasystole and gradually disappear. In addition, phase 4 and postextrasystolic AP changes are more marked in low Ca++ and less apparent in high Ca++. It would thus appear that the recovery period after an AP, i.e., electrical restitution, may be a manifestation of a transient phase in which the membrane undergoes changes in some way dependent on Ca++ ions. One must therefore consider what these membrane changes might be.

It is now generally agreed that a slow Ca++ current flows inward across the membrane during the plateau phase of the AP in frog atrium (23) and in mammalian ventricular (2, 17, 21) and Purkinje tissue (22, 25). It has also been established that Ca++ is an important component of the cell membrane and that changes in membrane-bound Ca++ are accompanied by changes in several physicochemical characteristics of the membrane (see ref. 16 for refs.). Little is known about the role that membrane-bound Ca++ might play in the inward flow of Ca++ ions during the AP plateau, but it is not unreasonable to assume that it might be in some way related.

The strongest evidence suggesting that such an assumption may be correct is the well-known observation (12, 14), also noted in the present study, that high Ca++ shortens the plateau duration while low Ca++ lengthens it. Since membrane Ca++ content presumably is elevated in high Ca++ and lowered in low Ca++, and since increased Ca++ conductance occurs during the plateau, an elevated membrane Ca++ content would be associated with a shorter period of inward Ca++ current and a lowered membrane Ca++ content with a prolonged Ca++ current.

Although highly speculative, one possible sequence of events responsible for phase 4 of electrical restitution, based on the above observations, might be as follows: an AP would trigger the release of Ca++ from intracellular storage sites. Some Ca++, an unknown fraction of the whole, would also be released into the sarcomeres from the cell membrane. If an extrasystolic AP were then interpolated shortly after repolarization of the control AP, and before the released Ca++ had returned to the membrane and restored the pre-AP level of membrane Ca++, the AP would have a more positive and prolonged plateau and greater area (i.e., resemble a low Ca-AP) because the amount of membrane-bound Ca++ would still be low. Exaggeration of these changes in low Ca++ and their attenuation in high Ca++ would be expected.

The inverse relationship between extrasystolic and postextrasystolic AP's can be explained on this same basis. The prolonged and large AP would be associated with a prolonged inward Ca++ current. Thus the Ca++ stores, including the membrane, would be loaded with additional Ca++, when an AP occurred, and the AP should resemble a high-Ca++ AP, which it does. Augmentation of the normal situation by low Ca++ and attenuation by high Ca++ again is to be expected. In the former case, the extraordinarily prolonged extrasystolic AP would be associated with (relative to the control AP) a larger and more prolonged Ca++ current and would be followed by a proportionally greater elevation of the membrane Ca++ content. Hence the next AP, AP should and does have a smaller area and shorter phase 2 than usual (relative to the control AP).

The gradual return of postextrasystolic AP's to control suggests that the increment of extra Ca++ contributed by the AP overload the membrane (and other Ca++ storage sites), and three to four AP's are required to return to equilibrium. This is indeed to be expected, since the occurrence of an extra ("extrasystolic") AP would not only admit an extra load of Ca++ into the cell, but also the extra increment of Ca++ would be made even larger by the phase 2 prolongation and large area of the AP. Minimal postextrasystolic AP changes in high Ca++ would also be expected if these changes were due originally to an elevation of Ca++.
extend the interpretation of Wood et al. by suggesting the additional presence of an AP-AP negative feedback loop, which maintains a constancy of the membrane Ca\(^{++}\) content over a period of several AP's. This feedback loop would be a consequence of the inverse relationship between membrane Ca\(^{++}\) content and the duration of the inward Ca\(^{++}\) current (and thus the AP plateau) suggested above. A lower membrane Ca\(^{++}\) content would result in a longer and greater inward Ca\(^{++}\) current; the greater number of Ca\(^{++}\) ions crossing the membrane in turn would elevate the membrane Ca\(^{++}\) content and tend to reverse the process in the next (or next few) AP's. Thus the area and plateau duration of any nongraded AP would depend (inversely) on the area and plateau duration of the AP which preceded it. Extra-systolic and postextrasystolic AP changes, as noted above, can be explained in terms of this control loop. A similar type of negative feedback loop subserving the same control function, but interpreted in terms of a different mechanism, also was described by Braveny and Sumbera (7). Slator and de Gubareff (24) studied the dependency of the duration of human and chimpanzee atrial muscle AP's on Ca\(^{++}\) and felt that their observations also could be explained best if AP duration were inversely proportional to the amount of membrane-bound Ca\(^{++}\).

Brady (5) has proposed a negative feedback mechanism by which the membrane Ca\(^{++}\) content might be responsible for the presence of the plateau and the repolarization of individual AP's. However, the feedback loop proposed by Brady operates during a single AP: the Ca\(^{++}\) influx associated with depolarization is assumed to lower the membrane Ca\(^{++}\) content, which in turn would result in a fall in K\(^{+}\) conductance and a transmembrane potential further from (more positive than) the K\(^{+}\) equilibrium potential. A longer period of depolarization (the plateau) and an associated greater influx of Ca\(^{++}\) ions would then occur, raising the membrane Ca\(^{++}\) content again, returning the K\(^{+}\) conductance to control and resulting in repolarization. Repolarization in such a scheme would be complete only when the Ca\(^{++}\) sink is filled. It would thus follow that the membrane Ca\(^{++}\) content must return to predepolarization level by the end of the AP. The hypothesis proposed in the present report suggests that the membrane Ca\(^{++}\) content does not return to predepolarization level by the end of the AP. Indeed, if correct, the end of phase A would be an approximate measure of the time after an AP required for membrane-bound Ca\(^{++}\) to reach its final stable resting level. Since Ca\(^{++}\) current flows only at transmembrane potential levels more positive than about 35 mV (in dog ventricular muscle (3)), and thus essentially only during the AP plateau, it is clear that membrane Ca\(^{++}\) rebinding would occur subsequent to the end of the Ca\(^{++}\) current and would not be directly coupled to it.

**Relationship between phase A of electrical restitution and phase I of mechanical restitution.** If phase A is indeed the obverse of phase I, as the observations in this investigation suggest, then derivation of the electrical and mechanical restitution curves from simultaneous AP and tension recordings should reveal an approximate concurrence of phases A and I. Figure 12 shows that this is the case. Unfortunately, because restitution curves are drawn by visual best fit, the imprecision inherent in the method does not permit a precise correlation between points on the respective curves. In addition, AP characteristics change independently. For example, dV/dt returns to normal at the peak of phase A of the restitution curve of AP area. Therefore, a precise correlation would not really be meaningful. Nevertheless, phases A and I clearly occur at approximately the same time in the cycle. If the interpretation of the meaning of phases A and I is correct, then the slopes of phase A and of phase I as they return to their respective restitution curves (Fig. 12, ef and yz slopes) are measures, however inexact, of the approximate time courses of Ca\(^{++}\) rebinding to the membrane and of its obverse, the return to rest level of the elevated free [Ca\(^{++}\)] in the sarcoplasm during a single cycle of electromechanical activity.

The present study was undertaken in part to determine whether changes in AP's played any causative role in mechanical restitution, especially during phase I. The results of the study indicate that, except possibly during the very beginning of phase I (see below), they do not. During the enhanced phases of electrical and mechanical restitution, low Ca\(^{++}\) exaggerates phase A but depresses phase I, while high Ca\(^{++}\) does the reverse. The change in opposite directions of phases A and I when the [Ca\(^{++}\)] is varied strongly implies the absence of any causative relationship between phases A and I. The observations can be explained more satisfactorily by considering the enhanced phases of both electrical and mechanical restitution to be causally related instead to the same intracellular change, rather than to each other. AP changes also play no role in the subsequent course of mechanical restitution, since AP's regain normal control characteristics by the end of phase A, which occurs approximately concurrently with the "dip" in the M-R curve (Fig. 19, point z). AP's remain normal and unchanged during the subsequent rapid and final slow phases of mechanical restitution.

An exception to the general absence of any causative relationship between electrical and mechanical restitution might occur during the very earliest part of phase I. It was...
suggested previously (1) that the rapid increase in tension during the $xy$ slope of the mechanical restitution curve might be associated in part with the disappearance of graded AP's (de slope of the electrical restitution curve). This would be difficult to demonstrate because the vague term graded refers to a complex of characteristics, including slow dV/dt, short plateau, small area, etc., and, as noted, these AP characteristics change independently. Thus one cannot tell exactly when graded AP's begin and end. Nevertheless, the $de$ and $xy$ slopes were concurrent and steep under all conditions studied. One might therefore speculate that if most of the Ca++ released by an AP is released during AP phase 0, a progressively faster and greater Ca++ release by AP's with progressively faster dV/dt and greater amplitude during this earliest phase of restitution could be a possible mechanism underling a dependency of tension on the AP at this time.

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