The Role of Calcium in Overdrive Suppression of Canine Cardiac Purkinje Fibers

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SUMMARY. The role of calcium in the electrical and mechanical changes induced by overdrive was studied in canine cardiac Purkinje fibers. The following results were obtained: (1) hyperpolarization and pause induced by a "short" (1-minute) overdrive are smaller in a low and larger in a high calcium solution; (2) overdriving at a constant ratio of overdrive to spontaneous rate only reduces the difference in pause duration in different calcium concentrations; (3) after overdrive, the initial diastolic depolarization is flatter and the late diastolic depolarization is steeper in low calcium and the opposite changes occur in high calcium; (4) the threshold for the first beat after overdrive is more negative in low than high calcium; (5) with "long" (2-minute or more) overdrives, hyperpolarization of comparable magnitude is seen during overdrive in low and high calcium; (6) the decline of hyperpolarization after overdrive is faster in high calcium unless the fiber is driven at slow rate ("postdrive"); (7) the contractile force after overdrive is greater than before overdrive in low and normal calcium and smaller than before overdrive in high calcium, and this difference is less during postdrive; (8) hyperpolarization and pause are reduced in a low sodium solution. It is concluded that calcium modifies overdrive suppression in several respects (hyperpolarization, slope of diastolic depolarization, duration of the pause and threshold), but is only one of the several factors involved in determining overdrive hyperpolarization and suppression. (Circ Res 51:167-180, 1982)

WHEN Purkinje fibers are driven at a rate faster than the spontaneous rate, the cessation of the drive is followed by a temporary suppression of spontaneous discharge ["overdrive suppression" (Vassalle et al., 1967a, 1967b; Alanis and Benitez, 1967; Vick, 1969; Vassalle, 1970; Browning et al., 1979)]. As to the mechanism of the changes induced by overdrive, an electrogenic extrusion of sodium appears to play a major role (Vassalle, 1970; Browning et al., 1979; Diacono, 1979; Courtney and Sokolove, 1979; Pelleg et al., 1980). However, other factors (see Vassalle, 1977) may contribute to overdrive suppression. In cardiac cells, an increase in driving rate increases calcium exchange (Niedergerke, 1963; Grossman and Furchgott, 1964b), the calcium liberated during the twitch (Allen and Blinks, 1978), and the calcium content (Sands and Winegrad, 1970). Therefore, calcium also should be considered as a possible factor in overdrive suppression, and this for several reasons. In cardiac Purkinje fibers, an increase in [Ca\textsubscript{i}] increases potassium conductance (g\textsubscript{K}) (Isenberg, 1977a, 1977b), and an increase in [Ca\textsubscript{o}] increases the background potassium conductance (Kass and Tsien, 1976) and potassium movements in active fibers (Musso and Vassalle, 1978). In fact, in Purkinje fibers, verapamil increases the rate of discharge and shortens the duration of overdrive suppression (Hogan and Spitzer, 1975). Thus, calcium could play a role in overdrive suppression by increasing potassium conductance. However, the changes induced by verapamil could be mediated to some undetermined extent by a change in the rate of spontaneous discharge of the fibers. If the spontaneous rate varies, overdrive at a fixed rate results in a suppression (the "pause") of different duration (Krellenstein et al., 1978).

These considerations suggest the need of testing the role of calcium in overdrive suppression, and the present experiments were done in response to such need. By varying the external calcium and sodium concentrations, it was sought to determine which electrophysiological features of overdrive suppression are affected. For example, if the overdrive hyperpolarization is related only to calcium-induced increase in g\textsubscript{K}, such a hyperpolarization should be reduced by low [Ca\textsubscript{o}]. If, instead, the electrogenic sodium extrusion is the only mechanism involved in overdrive suppression, the decrease in g\textsubscript{K} in low calcium would enhance the hyperpolarization. Reciprocally, by increasing g\textsubscript{K}, high calcium could initially enhance directly overdrive hyperpolarization and then reduce the hyperpolarization caused by an electrogenic sodium extrusion. In some experiments the contractile force was also recorded, as this gives useful information on the intracellular calcium.

A report of the results obtained has appeared in abstract form (Vassalle and Musso, 1978).

Methods

Mongrel dogs of either sex, weighing 12–20 kg, were anesthetized with sodium pentobarbital (30 mg/kg, iv). Strands of Purkinje fibers (1 mm or less in diameter) were removed from both ventricles and superfused in oxygenated (97% O\textsubscript{2} and 3% CO\textsubscript{2}) warm (37°C) Tyrode's solution.

The composition of the Tyrode's solution in mm was as
follows: NaCl, 137; KCl, 2.7; NaH₂PO₄, 0.45; NaHCO₃, 11.9; MgCl₂, 0.5; CaCl₂, 2.7; dextrose, 5.5. In a number of experiments, the calcium concentration was decreased (0.54 mM) or increased (5.4–8.1 mM). A low sodium solution was prepared by substituting half of the sodium with choline chloride (Sigma Chemical Co.). Atropine sulfate (Sigma Chemical Co., 10 mg/liter) was added to the sodium-poor choline solution. When norepinephrine (Levophed Bitartrate; Winthrop Laboratories) was used, its solution was prepared immediately prior to the experiment.

The preparations were either spontaneously active or driven. When driven, the stimuli were delivered to the Purkinje fibers by a Grass S4KR Stimulator via a Grass 4A Stimulus Isolation Unit and stainless steel pins electrically insulated except for the tip. The characteristics of the stimuli were: frequency 15 to 216/min, duration 2 msec, and voltage 20–40% above threshold. The membrane potentials were recorded by means of glass microelectrodes filled with 3 M KCl. The recording apparatus consisted of a push-pull cathode follower stage, a direct coupled Tektronix 3A3 dual trace differential amplifier, and a Tektronix RM565 dual beam oscilloscope.

In a number of experiments, the mechanical activity was recorded by fixing one end of the Purkinje strand with one of the stimulating electrodes and by attaching the other end to a rigid rod of a force displacement transducer (Grass model FTO 3C) by means of a short silk thread. The preparation (driven at 60/min) was kept at a length at which the contractile force was 60% of the maximal value. Electrical and mechanical events were displayed on the oscilloscope and were photographed on film with a Grass Kymograph camera model C4C.

The decrease and the increase in maximum diastolic potential (Eₘₘₖ) during or after overdrive with respect to the value before the overdrive will be referred to as depolarization and hyperpolarization, respectively. The threshold potential was measured by determining the intercept of two lines: one line was extrapolated from the latter part of diastolic depolarization and the other was extrapolated downward from the potential curve immediately preceding the upstroke. The potential value thus obtained was expressed in mV with respect to the maximum diastolic potential of the preceding action potential. A shift of the threshold potential to a more positive value will be referred to as an increase in the threshold potential (more depolarization to induce activity).

The diastolic depolarization has been distinguished into two phases (initial and late phase) which have a different slope, especially after overdrive.

**Results**

**Effects of a “Short” Overdrive in Different [Ca].**

In a first series of experiments (Fig. 1), 1-minute overdrive at 90/min was carried out in Tyrode’s solution containing a normal (A), low (B), and high (C)
calcium concentration. In A, after four spontaneous action potentials (rate 20/min), the drive was initiated. The maximum diastolic potential decreased during the first 30 seconds of drive and then slowly increased again. After the cessation of the drive, the slope of the initial diastolic depolarization was depressed with respect to predrive and declined during the late phase much more slowly. An oscillatory prepotential preceded the resumption of spontaneous activity after a 27-second pause. Typically, the resumed spontaneous activity was characterized by a progressive steepening of diastolic depolarization and a progressive increase in the rate of discharge toward the control value. In B, superfusion with a low Ca solution resulted in an increase in rate of discharge (to 26/min) due to a shift of the threshold to a more negative value. During overdrive, $E_{\text{max}}$ decreased as usual but did not increase again. After the drive, the slope of the initial phase of diastolic depolarization was flatter than prior to the drive, but that of the late phase was steeper and quickly attained the threshold: as a result, the pause was rather short (9 seconds). As in normal Tyrode’s solution, the slope of diastolic depolarization increased progressively with each spontaneous action potential as the preparation accelerated toward its control value. At the same time, $E_{\text{max}}$ increased above the values recorded prior and during the drive. It should be noted that the slope of the initial diastolic depolarization after drive was flatter than that in normal calcium (cf. A).

In high calcium (C), the spontaneous rate was slower (14/min) due to a flattening of the late diastolic depolarization and to a more positive threshold. During the drive, $E_{\text{max}}$ fell very little at the beginning and progressively increased during the drive. After the drive, the slope of the initial diastolic depolarization was faster than prior to the drive (and also than in A and B). The slope of the late diastolic depolarization was fairly flat and the pause long (63 seconds).

The results obtained in six experiments are reported in Table 1 and show that, in general, the changes in low and high calcium mirror each other by shifting in opposite direction with respect to normal calcium solution. The points of major interest in the present context are that the hyperpolarization was less marked and the pause was shorter in low calcium solution, whereas the opposite changes occurred in high calcium solution. However, it should be pointed out that the hyperpolarization after the drive occurred also in low calcium (compared to the value at the end of the drive, the hyperpolarization was almost as large as in normal calcium); that the hyperpolarization in high calcium sometimes began immediately with drive; and that the hyperpolarization in high calcium decreased more quickly after the drive (and was not greater than at the end of the drive).

**Overdrive Suppression at a Constant Overdrive: Spontaneous Rate Ratio**

In the experiments reported above, the overdrive rate was constant but the spontaneous rate was not. Therefore, the overdrive was relatively larger when the spontaneous rate was slower, and this should have influenced the duration of the pause to some undetermined extent. For this reason, the same preparations were overdriven at a constant multiple (X3) of the spontaneous rate. In this manner, the only variable under study was the calcium concentration.

### Table 1

Effects of Low and High Calcium on 1 min Overdrive at 90/min

<table>
<thead>
<tr>
<th>Ca = 2.7 mm</th>
<th>Ca = 0.54 mm</th>
<th>Ca = 5.4 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rate (beats/min)</strong></td>
<td>Avg ± SE</td>
<td>Avg ± SE</td>
</tr>
<tr>
<td>DDi, prior (mV/sec)</td>
<td>19.0 ± 3.2</td>
<td>31.3 ± 4.3*</td>
</tr>
<tr>
<td>Threshold prior (mV)</td>
<td>6.4 ± 1.2</td>
<td>5.6 ± 1.3</td>
</tr>
<tr>
<td>Depol. during (mV)</td>
<td>12.4 ± 1.3</td>
<td>7.5 ± 0.7*</td>
</tr>
<tr>
<td>Hyperpol. end (mV)</td>
<td>1.0 ± 0.2</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Max. hyperpol., after (mV)</td>
<td>1.1 ± 0.4</td>
<td>-0.3 ± 0.2*</td>
</tr>
<tr>
<td>DDi after (mV/sec)</td>
<td>3.7 ± 0.4</td>
<td>1.9 ± 0.3*</td>
</tr>
<tr>
<td>Hyperpol. end (mV)</td>
<td>4.6 ± 1.2</td>
<td>3.5 ± 1.0*</td>
</tr>
<tr>
<td>Max. hyperpol., after (mV/sec)</td>
<td>0.9 ± 0.5</td>
<td>2.7 ± 1.2</td>
</tr>
<tr>
<td>Threshold after, 1st beat (mV)</td>
<td>16.1 ± 1.8</td>
<td>12.0 ± 1.7*</td>
</tr>
<tr>
<td>Pause (sec)</td>
<td>19.0 ± 5.0</td>
<td>6.1 ± 1.3*</td>
</tr>
</tbody>
</table>

Ca = 2.7, 0.54, 5.4 mm: results obtained in the fibers superfused with solutions containing normal (2.7), low (0.54), and high (5.4 mm) calcium, respectively. Avg. ± se: average values and standard error. Rate (beats/min): spontaneous rate of the preparations in beats/min. DDi, prior (mV/sec): initial diastolic depolarization prior to overdrive in mV/sec. Threshold prior (mV): threshold potential measured as the difference in mV between maximum diastolic potential and the threshold, prior to overdrive. Depol. during (mV): largest depolarization in mV during overdrive. Hyperpol. end (mV): hyperpolarization in mV at the end of overdrive. Max. hyperpol., after (mV): maximal increase in $E_{\text{max}}$ in mV after the end of overdrive. DDi, after (mV/sec): initial diastolic depolarization of the last overdriven action potential. DDi, after (mV/sec): late diastolic depolarization following drive. Threshold after, 1st beat (mV): threshold potential measured as above for the first spontaneous beat after overdrive. Pause (sec): duration of the pause in seconds.

* P value < 0.05-0.001 with respect to control (Ca = 2.7 mm).
† P value < 0.05-0.001 for the difference between low and high calcium solutions.
The average results in six experiments are reported in Table 2. It is apparent that the durations of the pauses in Tables 1 and 2 are different, particularly in high calcium solution. The larger difference in the higher calcium solution would be expected since the spontaneous rate was lowest. As shown in Table 2, the usual changes during and after overdrive were noted in the different calcium solutions. If these experiments show that the ratio of overdrive to spontaneous rate influences the pause, they also show that the difference in pause duration persisted even if such ratio was kept constant.

**Effects of a “Long” Overdrive at Different [Ca]<sub>o</sub>**

In low calcium, E<sub>max</sub> did not increase during overdrive, but it increased after the drive. This finding suggests that hyperpolarization during overdrive might occur even in low calcium, provided the over-
drive period is sufficiently long. In Figure 2, a spontaneous preparation superfused in low calcium solution was overdriven at 3 times the spontaneous rate for 30 seconds (A), 2 minutes (B), and 4 minutes (C). With the shortest overdrive, $E_{\text{max}}$ decreased during and increased after the drive. With the longer drives, $E_{\text{max}}$ decreased initially, as usual, but at the end of the drive was more negative than prior to the drive (B) and more so with the longest drive (C). After the overdrive, the usual changes (such as a flattening of the late depolarization, a gradually longer pause, and a more positive threshold) were also present. Also, $E_{\text{max}}$ of the resumed beats was initially similar to the value at the end of the drive and declined subsequently toward the control value, as did the threshold and the diastolic depolarization.

In Figure 3, the same preparation was overdriven at 3 times the spontaneous rate in high calcium for 30 seconds (A), 2 minutes (B), and 4 minutes (C). With the shortest overdrive, $E_{\text{max}}$ increased progressively from the first beat and, at the same time, the action potential became shorter and diastolic depolarization steeper. With the longer drives, $E_{\text{max}}$ increased during the overdrive and more so the longer the drive. After the overdrive, the usual changes (such as a steepening of the initial diastolic depolarization, a flattening of the late diastolic depolarization, a gradually longer pause, and a somewhat more positive threshold) were present. Oscillatory pre-potentials were present before the resumed spontaneous activity and became progressively larger (see the fourth, fifth and sixth spontaneous beat in each strip) leading to the resumption of the regular spontaneous discharge. $E_{\text{max}}$ of all resumed beats was less negative than at the end of drive.

In Figure 4, superimposition of the records at the end of the 4-minute drive in low (Fig. 2C) and high (Fig. 3C) calcium solution show that the degree of hyperpolarization at the end of drive was similar in the two solutions, and that, in low calcium during the pause, the initial depolarization was slower and the late depolarization steeper than in high calcium. Also, in low calcium, the threshold was more negative and the transition from diastolic depolarization to the action potential upstroke faster than in high calcium. During recovery, $E_{\text{max}}$ fell more gradually in low than in high calcium. The importance of the threshold shifts in determining the duration of the pause in the two calcium solutions is suggested by the fact that if the threshold had been the same, the pause would have been shorter in high calcium.

A 4-minute overdrive was carried out under similar

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Effect of overdrive of different duration in the presence of high calcium (5.4 mM). The overdrive lasted 30 seconds in A, 2 minutes in B, and 4 minutes in C. In B and C, only the beginning and the end of overdrive are shown.
conditions in three spontaneous (16 ± 6.2 beats/min) fibers. In addition to the usual changes, the following results were obtained in the normal, low, and high calcium solutions, respectively: depolarization during overdrive 0.5 ± 0.1, 0.8 ± 0.4, and 0.0 mV; hyperpolarization at the end of overdrive 5.7 ± 0.6, 6.5 ± 1.3, and 4.7 ± 1.7 mV; maximal hyperpolarization after overdrive 4.3 ± 1.8, 7.1 ± 1.3, and 3.5 ± 0.5 mV. An 8-minute overdrive was carried out in two experiments in low and high calcium with the following results, respectively: depolarizations during overdrive 1.2 and 0.0 mV; hyperpolarization at the end of overdrive 8.2 and 5.6 mV; maximal hyperpolarization after overdrive 8.6 and 5.2 mV.

In addition, in five experiments, the preparations were predriven at a constant rate (39.6 ± 4.1/min) and overdriven at 3 times the control rate for 2 minutes in normal, low, and high (8.1 mM) calcium solution (Table 3). Many of the results show the usual trends, in particular: (1) there was a hyperpolarization at the end of 2-minutes drive in low calcium; and (2) hyperpolarization became somewhat larger in low than in normal calcium and equal to that in high calcium after the drive. Thus, as the duration of the drive is increased in low calcium, the hyperpolarization eventually becomes equal or greater than in either normal or high calcium.

Because the initial diastolic depolarization became steeper with longer overdrives in high calcium, in one experiment the duration of the drive was kept constant (4 min) and, instead, the rate was increased in successive tests (Fig. 5) in the presence of normal (2.7 mM) calcium (lefthand panels) and of high (8.1 mM) calcium (righthand panels). Overdrive was carried out at 3, 5, and 10 times the predrive rate, as indicated in the left hand side of the figure. In the normal calcium solution, an increase in the frequency of the drive changed little the initial phase of diastolic depolarization but shifted the late phase to more negative values. In the high calcium solution, instead, there was a clear oscillatory afterpotential superimposed on the initial diastolic depolarization which became faster

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Ca = 2.7 mM</th>
<th>Ca = 0.54 mM</th>
<th>Ca = 8.1 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avg ± se</td>
<td>Avg ± se</td>
<td>%Δ</td>
</tr>
<tr>
<td>$D_{Di}$, prior (mV/sec)</td>
<td>5.1 ± 0.9</td>
<td>4.5 ± 1.2</td>
<td>-11.8</td>
</tr>
<tr>
<td>Threshold prior (mV)</td>
<td>6.9 ± 0.8</td>
<td>4.8 ± 0.4*</td>
<td>-30.5</td>
</tr>
<tr>
<td>Depol. during (mV)</td>
<td>0.7 ± 0.1</td>
<td>1.4 ± 0.3*</td>
<td>+100.0</td>
</tr>
<tr>
<td>Hyperpol. end (mV)</td>
<td>2.2 ± 0.1</td>
<td>1.6 ± 0.3</td>
<td>-27.3</td>
</tr>
<tr>
<td>Max. hyperpol. (mV)</td>
<td>2.4 ± 0.06</td>
<td>2.8 ± 0.6</td>
<td>+16.6</td>
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<tr>
<td>$D_{Di}$, after (mV/sec)</td>
<td>4.3 ± 0.6</td>
<td>3.3 ± 0.7</td>
<td>-23.3</td>
</tr>
<tr>
<td>$D_{Di}$, after (mV/sec)</td>
<td>0.1 ± 0.2</td>
<td>0.7 ± 0.4</td>
<td>+600.0</td>
</tr>
<tr>
<td>Threshold after, 1st beat</td>
<td>23.0 ± 2.1</td>
<td>25.5 ± 13.0*</td>
<td>-70.2</td>
</tr>
<tr>
<td>Pause (sec)</td>
<td>85.6 ± 16.6</td>
<td>25.5 ± 13.0*</td>
<td>-70.2</td>
</tr>
</tbody>
</table>

Explanation as for Table 1. The values for the pause include a pause of 120 seconds (after which drive was resumed) in one experiment in normal calcium and in three experiments in high calcium.
Musso and Vassalle/Calcium and Overdrive Suppression

and smaller after the fastest drive. Also, after the oscillatory potential, the late phase of diastolic depolarization returned to values similar to those seen in Tyrode’s solution. The findings are consistent with the fact that the steepening of the initial diastolic depolarization after overdrive in high calcium (Temte and Davis, 1967) results from a superimposed oscillatory afterpotential (see Vassalle and Mugelli, 1981).

Effects of Low-Sodium Solutions

To gain more information on the relative importance of sodium vs. calcium in overdrive suppression, the extracellular sodium concentration was decreased with and without a concomitant decrease in [Ca]o. A decrease in [Na]o results in an increase in [Ca]i (Reuter and Seitz, 1968; Baker et al., 1969). Thus, the hyperpolarization and the pause may be reduced or enhanced, depending on whether sodium or calcium is the more important factor.

In Figure 6, the lower part of the action potential and the twitch of a preparation predriven at 30/min and then overdriven at 90/min for 4 min in Tyrode’s solution (A), in low sodium solution (74.6 mM, B) and low Na (74.6 mM) plus low calcium (0.73 mM, C) are shown. In each strip, overdrive begins in the first panel and ends in the second panel. In Tyrode’s solution, the usual electrophysiological features associated with overdrive are apparent. The preparation was quiescent for 120 seconds after the overdrive and, after that interval the 30/min drive (not shown) was resumed. In B, perfusion with a low Na solution resulted in a steepening of diastolic depolarization and an increase in force of contraction (+50%). It is possible that the steepening of the diastolic depolarization was due to a superimposed oscillatory afterpotential, since the pacemaker current should be reduced in low sodium (McAllister and Noble, 1966; see below). In this case, there was no hyperpolarization by the end of drive: the slope of diastolic depolarization during the pause was more pronounced than in A and the maximal diastolic potential and contractile force of the resumed beats were less than prior to overdrive. In C, the slope of diastolic depolarization and the contractile force of the resumed beats were greater than prior to the drive (see below).

In five experiments, the fibers were predriven at 30/min and overdriven at 90/min for 4 minutes. The contractile force was greater (+29.0 ± 31.3%) in low sodium solution, as would be expected on the basis of an increased [Ca]i. The hyperpolarization at the end of the drive was less in the low sodium than in Tyrode’s solution (−71.6 ± 8.0%, P < 0.02). The pause was 112.7 seconds in the low-sodium solution and more than 120 seconds in Tyrode’s solution (the 30/min drive was resumed at the end of this period). The initial diastolic depolarization after overdrive was faster in low sodium than in Tyrode’s solution (±168.4 ± 69.4%). In the low Na plus low Ca solution (n = 7), the force decreased (−30.3 ± 9.0%) and so did the slope of the initial diastolic depolarization (−9.0 ± 13.6%) with respect to Tyrode’s solution. The initial depolarization during overdrive was less (−26.6 ± 27.8%) and the hyperpolarization at the end was more (+35.1 ± 26.6%) than in Tyrode’s solution. After overdrive (n = 5), the initial diastolic depolarization was less (−36.5 ± 10.1%; P < 0.02) but (due to the faster late diastolic depolarization, +1114.2 ± 708.9%), the pause was shorter (−67.8 ± 12.7%; P < 0.01).

The experiments show that the hyperpolarization and the pause were reduced when [Na]o was less and [Ca]i was presumably increased, and persisted when the external calcium was reduced together with the sodium.

Effect of Norepinephrine on the Changes Induced by Overdrive

Catecholamines decrease the duration of the pause (Kellihé et al., 1972; Pliam et al., 1975; Pliam et al., 1977) through an undetermined mechanism. In view of the fact that catecholamines increase calcium influx (Grossman and Furchgott, 1964c; Reuter, 1965) as well...
Figure 6. Effects of low sodium on overdrive suppression. The preparation was predriven at 30/min and overdriven at 90/min for 4 minutes in Tyrode’s solution (A), in a low (74.6 mM) sodium (B), and in a low sodium plus low calcium [(74.6 and 0.73 mM, respectively) (C)] solution. In each panel, the top trace shows the lower part of the action potential and the bottom trace a record of the contractile force. The initiation and cessation of overdrive is indicated above A.

as stimulate the sodium potassium pump (Vassalle and Barnabei, 1971; Borasio and Vassalle, 1974; Lee and Vassalle, 1982), the shortening of the pause must involve other mechanisms. The average results in normal, low, and high calcium solutions are shown in Table 4 (n = 5). In each experiment, the preparations were driven at a fixed rate and also at 3 times the spontaneous rate: as the effects of norepinephrine were similar, the results were pooled together. It is apparent that norepinephrine (10^{-7} M) enhanced the rate of discharge, the slope of the initial and late diastolic depolarization before and after overdrive, and the hyperpolarization during overdrive (in normal and low calcium solution). It appears, therefore, that the shortening of the pause is due to the fact that overdrive depresses the slope of diastolic depolarization in the absence and in the presence of norepinephrine, but less so in the latter case. The one

Table 4
Effects of NE at Different [CA], (1 min Overdrive)

<table>
<thead>
<tr>
<th></th>
<th>Ca 2.7 mM</th>
<th>Ca 0.54 mM</th>
<th>Ca 5.4 mM</th>
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<tbody>
<tr>
<td></td>
<td>Contr</td>
<td>NE</td>
<td>%Δ</td>
</tr>
<tr>
<td>Rate (beats/min)</td>
<td>19.4 ± 2.3</td>
<td>31.0 ± 2.6*</td>
<td>+59.8</td>
</tr>
<tr>
<td>DD1 prior (mV/sec)</td>
<td>6.3 ± 1.0</td>
<td>11.4 ± 2.4*</td>
<td>+80.9</td>
</tr>
<tr>
<td>DD2 prior (mV/sec)</td>
<td>4.8 ± 0.7</td>
<td>6.3 ± 1.1*</td>
<td>+31.3</td>
</tr>
<tr>
<td>Hyperpol. end (mV)</td>
<td>1.1 ± 0.3</td>
<td>2.0 ± 0.7</td>
<td>+81.8</td>
</tr>
<tr>
<td>Max. hyperpol. (mV)</td>
<td>3.1 ± 0.3</td>
<td>3.5 ± 0.8</td>
<td>+12.9</td>
</tr>
<tr>
<td>DD1 after (mV/sec)</td>
<td>5.0 ± 0.9</td>
<td>8.8 ± 1.9*</td>
<td>+76.0</td>
</tr>
<tr>
<td>DD2 after (mV/sec)</td>
<td>1.3 ± 0.3</td>
<td>1.6 ± 0.4</td>
<td>+23.1</td>
</tr>
<tr>
<td>Pause (sec)</td>
<td>39.9 ± 16.9</td>
<td>21.3 ± 9.0</td>
<td>-46.6</td>
</tr>
</tbody>
</table>

NE results obtained in the presence of 10^{-7} M norepinephrine. Other explanations as for Table 1.
FIGURE 7. Electrical and mechanical events associated with overdrive. The top trace shows the lower part of the action potential. The bottom trace shows the twitch tension recorded simultaneously. The fiber was predriven at 48/min and overdriven at 144/min for 2 minutes.

notable exception is the high calcium solution where norepinephrine actually increased the slope of the initial diastolic depolarization above the control value.

Similar results were obtained with a 2-minute drive when norepinephrine was given in Tyrode's solution (n = 6), in low calcium (n = 6) and in high calcium (n = 4).

Overdrive induced similar changes in force of contraction in the absence and in the presence of norepinephrine (n = 4), but the absolute values were consistently higher in the presence of norepinephrine.

**Effect of Drive on Contractile Force**

Because changes in contractile force with overdrive may reflect changes in cellular calcium, force was recorded in Tyrode's solution before, during, and after overdrive, as shown in Figure 7. The electrical events shows the usual changes. The contractile force decreased at the beginning of overdrive and increased progressively during drive without, however, attaining the control value. After the overdrive, there was a pause of 33 seconds. The resumption of activity was characterized by a transient increase of force above control.

To find out whether the same features occurred in different $[\text{Ca}]_o$, the same preparation was overdriven in the presence of low and high calcium (Fig. 8). In low calcium (A), the contractile force was far less than in normal calcium. During overdrive, the force initially decreased and subsequently increased somewhat without reaching the control value. After the drive, the contractile force increased above control with the first few resumed beats. In high calcium (B), the contractile force was greater than in Tyrode's solution. During overdrive, the contractile force fell initially and then increased progressively. After overdrive, the force was less than either before or at the end of the drive. It should be pointed out that, in high

FIGURE 8. Electrical and mechanical events associated with overdrive in low and high calcium. The traces recorded in low calcium (0.54 mM) are shown in A and those in high calcium (8.1 mM) are shown in B. Same preparation as in Figure 7.
calcium, often the contractile force fell minimally at the beginning of drive and was maintained during the drive. However, even then, the force decreased after the pause.

In four experiments, the force decreased by $-53.7 \pm 11.2\%$ in low calcium and increased by $147.2 - 56.4\%$ in high calcium (8.1 mM). At the beginning of overdrive in normal, low, and high calcium, respectively, the force decreases by $48.3 \pm 5.8$, $27.1 \pm 4.7$, and $32.3 \pm 10.5\%$; and at the end of overdrive, the force was $-10.0 \pm 6.2$, $-19.6 \pm 6.9$, and $+9.4 \pm 15.7\%$ with respect to control. The first beat after the pause was $+2.4 \pm 14.8$, $+67.8 \pm 54.4$, and $-36.8 \pm 2.1\%$ with respect to predrive value in each of the respective calcium solutions. Thus, during the recovery from overdrive, the force was more than the predrive value in low calcium but was less than the predrive value in high calcium.

**Effect of “Postdrive” on E\(\text{max}\) and Contractile Force**

The reason for the more rapid decline of E\(\text{max}\) and for the low force in high calcium during the recovery from overdrive is not apparent. One possibility is that both result from the longer pause and slower spontaneous rate in high calcium. This was tested by driving the preparations at a constant rate immediately after the end of overdrive in different calcium solutions. In Figure 9 (obtained from the same experiment as in Figs. 7 and 8), the preparation was “postdriven” in Tyrode solution (A), in low calcium (B), and in high calcium (C) at the same rate as prior to overdrive. It is apparent that during the “postdrive,” E\(\text{max}\) declined gradually in each of the three solutions. The difference in the absence and the presence of postdrive was most marked for the high calcium solution (cf. panels C in Figs. 8 and 9). The behavior of the contractile force with postdrive was also different. In Tyrode’s solution (A), contractile force was greater than control immediately after overdrive and then decreased gradually. In low calcium solution (B), the contractile force was similar to control and varied very little during postdrive. In high calcium (C), the force increased within a few beats to a value similar to control and then decreased somewhat thereafter. Similar results were obtained in two experiments. These results suggest that the fall in E\(\text{max}\) and in force in high calcium after overdrive are indeed due to the long period of quiescence, possibly because during quiescence the net extrusion of cellular calcium in the unit time is greater than during postdrive.

**FIGURE 9.** Effects of post-drive on the electrical and mechanical events recorded in normal, low and high calcium. The A panel was recorded in normal (2.7 mM), the B panel in low (0.54 mM), and C panel in high (8.1 mM) calcium solution. Same preparation as in Figures 7 and 8.
Discussion

The present experiments show that calcium markedly modifies the electrical and mechanical events induced by overdrive in Purkinje fibers. The experiments also show that the features of overdrive suppression persists when the role of calcium is minimized. Therefore, it would appear that calcium participates in the events underlying overdrive suppression as one of the several (see Vassalle, 1977) factors involved.

That calcium markedly influences the events associated with overdrive suppression is shown by the changes in the hyperpolarization and pause duration when the external concentration of calcium is varied. Since the changes were in opposite direction in low and high calcium, the inference is that calcium has some intermediate effects at normal concentration. The present findings are in line with those of Hogan and Spitzer (1975) who showed that in the presence of verapamil, the spontaneous rate was faster and overdrive caused a smaller hyperpolarization and a shorter pause. Furthermore, the present experiments show that when the changes in spontaneous rate are taken into account by overdriving at a multiple of the spontaneous rate, the differences in pause duration in different calcium solutions are smaller but still persist. These findings make it clear that calcium influences overdrive suppression directly as well as indirectly through a change in the spontaneous rate of discharge.

The Effect of Calcium on Overdrive-Induced Changes

In considering the action of calcium on overdrive suppression, one has to consider first the effects of varying the calcium concentration on the electrical activity of Purkinje fibers. A decrease in [Ca]o is known to shift the threshold to more negative values, increase the rate of spontaneous discharge (Weidmann, 1955), decrease the slope of diastolic depolarization, and decrease slightly the maximum diastolic potential (Temte and Davis, 1967). Increasing the extracellular calcium concentration has the opposite effects (Weidmann, 1955; Temte and Davis, 1967). The changes would affect overdrive suppression even if calcium movements were not modified by overdrive. However, there seem to be additional effects. Thus, during short drives, the hyperpolarization is less when the [Ca]o is lower and is more when [Ca]o is higher than in Tyrode's solution, suggesting that indeed calcium participates in overdrive hyperpolarization. In low calcium solution, there would be no reasons for the hyperpolarization to be less pronounced (or absent) if calcium played no role altogether. This is reinforced by the fact that the effect of high calcium can be immediate (increase in Emax from the beginning of drive), whereas the stimulation of the sodium pump is likely to be more gradual. It is possible then that calcium contributes to the suppression that follows short and long drives but might be more important for the suppression by short drive when the contribution of the pump is less.

The Mechanisms of Calcium-Induced Modifications

The participation of calcium in overdrive hyperpolarization elicits the question as to the mechanism of such calcium effect. One obvious possibility is that calcium influences the hyperpolarization by accumulating in the cell (including the cell membrane) during overdrive and thereby increasing potassium conductance. A calcium-induced increase in gK has been found in several tissues (see Romero and Whittem, 1971; Vassort, 1975), including cardiac Purkinje fibers (Isenberg, 1977a, 1977b; Kass and Tsien, 1976). In Purkinje fibers, high calcium has been shown to increase radioactive potassium movements associated with activity (Musso and Vassalle, 1978). Since EK is more negative than Emax at the usual [K]o, an increase in gK would result in hyperpolarization and contribute to the prolongation of the pause. Increasing the extracellular calcium concentration increases total cellular calcium (Niedegerke, 1963; Grossman and Furchgott, 1964a, 1964b), and increasing the driving rate increases calcium exchange (Grossman and Furchgott, 1964b) and also cellular calcium (Sands and Winegrad, 1970). Therefore, the membrane potential would be expected to increase during overdrive and more so when [Ca]o is higher. That this is a factor in hyperpolarization is shown by the fact that the hyperpolarization was absent with short drives in low calcium and was enhanced in high calcium (Fig. 1, Tables 1 and 2). Even more important, the hyperpolarization may begin at the very onset of overdrive in high calcium solution (Fig. 3); this is compatible with a progressive accumulation of calcium at the cell membrane as the number of action potentials in the unit time increases and the diastolic interval becomes shorter. It would seem less likely that the sodium pump should be significantly activated with the very first beats. Furthermore, an increase of [Na] during overdrive (Fozzard and Sheu, 1981) favors an increase of cellular calcium through the Na-Ca exchange (Reuter and Seitz, 1968; Baker et al., 1969; Fozzard and Sheu, 1981). That such accumulation of calcium occurs is strongly suggested by the transient enhancement of the twitch during the postdrive in Tyrode's solution (Fig. 9A). As the rate and [Ca]o are the same before and after the drive, an increase in contractile force can be explained by an increase in cellular calcium. This may result in an increased intracellular release of calcium during the action potential even if the slow inward current is unaltered (Fabiato and Fabiato, 1978). On the basis of these results, it would appear that, in Tyrode's solution, a calcium-induced increase in gK contributes to moderate the fall in Emax caused by extracellular potassium accumulation during short drives (Vassalle et al., 1967a, 1967b; Vassalle, 1970; Vassalle et al., 1977; Pliam et al., 1977; Kunze, 1977; Krellenstein et al., 1978; Kline and Morad, 1978; Kline et al., 1980). Also, the results suggest that the increase in gK induced by calcium (and by the extracellular potassium accumulation) may be important for the suppression induced by a few beats.
The pause was consistently shorter in low than in normal calcium, whether overdrive hyperpolarization was present (Fig. 2) or absent (Fig. 1). Factors other than hyperpolarization must be also important for the change in pause duration. The present experiments show that the slope of late diastolic depolarization is steeper in low than in normal or higher calcium solutions. As a consequence, less time is required to attain the threshold in low calcium. Ceteris paribus, a decrease in $g_K$ would result in an enhancement of the net inward current during the late diastolic depolarization. Another factor is shift of the threshold potential. After overdrive, the threshold did shift to a more positive value also in low Ca (Fig. 2), but the shift was less pronounced in low than in high calcium. Superimposition of the traces in Figure 4 shows that if the threshold in the high calcium had been the same as in the low calcium solution, the pause would have been far shorter in high calcium. In low calcium, then, a faster late diastolic depolarization attains sooner a more negative threshold: hence, the shortening of the pause. The opposite occurs in high calcium. Additional factors that may contribute to the same effects are the quick upswing of diastolic depolarization into the upstroke of the action potential and the lack of oscillatory prepotentials in the low calcium solution.

The Initial Diastolic Depolarization and Calcium

The slope of the initial diastolic depolarization is decreased in low and increased in high calcium (Temte and Davis, 1967; present results). The explanation for the effect of calcium on the early diastolic depolarization is that, between $E_{max}$ and the late diastolic depolarization, calcium induces an event which modifies the normal diastolic depolarization. This event is an oscillatory inward current which is triggered on repolarization and is superimposed on (and separable from) the pacemaker current (Vassalle and Mugelli, 1981). This oscillatory current is enhanced by conditions which increase cellular calcium, such as repetitive activity, higher $[Ca]_i$, norepinephrine, and strophanthidin (Vassalle and Mugelli, 1981). As this current peaks in about a second and then subsides, its effects on potential are seen only during the early diastolic depolarization. The characteristics of the oscillatory current readily account for the following findings: (1) the flattening of the initial diastolic depolarization in low calcium (Fig. 1), (2) the restoration of this component in low calcium by norepinephrine (Table 4), (3) the steepening of the early diastolic depolarization in the high calcium solution (Fig. 1), (4) the exaggeration of this phenomenon in high calcium after overdrive (Fig. 3), with faster drives (Fig. 5) and with norepinephrine (Table 4), and (5) a degree of steepening of the early diastolic depolarization observed in low sodium and flattening in the low sodium-low calcium solution.

This oscillatory afterpotential does not interfere with overdrive suppression because it occurs too late to affect $E_{max}$ (see Vassalle and Mugelli, 1981) and fails to attain the threshold. However, it may influence the late diastolic depolarization in that it rapidly brings the membrane potential to a value where the time constant of deactivation of the pacemaker current (Noble and Tsien, 1968) is reduced.

The Relative Importance of Calcium-Induced Increase in $g_K$ in Overdrive Hyperpolarization and Suppression

The question should be asked as to whether the calcium-induced increase in $g_K$ is the only or the major factor in overdrive hyperpolarization and suppression. Several findings suggest that it is not so, at least for longer drives: (1) hyperpolarization follows a short drive in low calcium solution (Figs. 1 and 2). There are no reasons for cell calcium to increase after a period of drive in low calcium solution, as the rate is slower than before the overdrive (Fig. 1) or is similar to that before overdrive (Fig. 2). Furthermore, (2) the hyperpolarization occurred also in low $[Ca]_i$ when the drive was prolonged to 2 minutes; (3) the hyperpolarization became progressively more pronounced when drive was continued beyond two minutes; (4) the hyperpolarization was as large in low calcium as in high calcium solution when the pause was still drastically different; (5) the hyperpolarization was larger in low than in high calcium solution with the longest overdrive; and (6) the pause became shorter in low Na solution (when cellular calcium is certainly increased). All these features are instead compatible with an electrogenic sodium extrusion which would be expected to continue past the cessation of overdrive, favored in its effects by a fall in $g_K$ induced by low calcium (as the shunting of the electrogenic sodium extrusion would be less). In addition, it has recently been shown that consecutive brief interruptions of the drive during the positive inotropic effect of strophanthidin reveal a progressively less negative diastolic depolarization and a progressively shorter pause (Vassalle and Ishikawa, 1980). Since, in the presence of strophanthidin, cellular calcium should increase (the force increases) and the sodium pump should be inhibited, the enhanced diastolic depolarization and the shortening of the pause suggests that an increasing calcium accumulation does not substitute for a declining pump activity in maintaining overdrive suppression. A similar situation obtains in the presence of metabolic inhibitors in that—during overdrive—the force still increases, but the hyperpolarization is reduced or absent (Bhattacharyya and Vassalle, 1980). Since the inhibition of metabolism increases cellular calcium (see Carmeliet, 1978), and presumably more so during overdrive, this also suggests that, in the absence of pump activation, calcium accumulation alone is insufficient to account for the phenomena underlying overdrive suppression.

It could be objected that with long drives calcium may progressively accumulate in the cells even in a low calcium solution, and therefore the hyperpolarization would appear and increase with the longer overdrives. While such a progressive accumulation is very probable (as the shift in threshold after overdrive
suggests), several findings make it unlikely that, quantitatively, the calcium accumulation is the major factor in the hyperpolarization and suppression. Thus, in low calcium solution: (1) the action potential during overdrive remained longer than at normal calcium; (2) the time course of the initial and late diastolic depolarization remained that seen in low calcium; (3) the pause remained shorter than in normal calcium; (4) $E_{\text{max}}$ fell slowly after overdrive (in contrast to the findings in high calcium solution); and (5) the contractile force did not increase above control during postdrive in the low calcium solution.

In considering the effects of calcium, it is realized that high calcium may not simply add its effects to those induced by an electrogenic sodium extrusion, but instead may modify that component. High calcium not only may reduce the effect of an electrogenic pump indirectly (increase in $g_K$) but also may inhibit the pump directly (Skou, 1957). Therefore, to some degree, high calcium can substitute its own effects on the membrane potential to those of the pump. This is suggested by the occurrence of an immediate increase in $E_{\text{max}}$ with overdrive (increase in $g_K$) and by the marked decrease of $E_{\text{max}}$ after the drive (depression of the pump, Figs. 3 and 8). During the long pause that follows overdrive, an elimination of the calcium accumulated during overdrive would account for the marked fall in $E_{\text{max}}$, especially if the sodium pump is inhibited by the high calcium. The inhibition of the pump by high calcium could contribute to the fact that the hyperpolarization during overdrive was less with longer drives in high calcium or in the low calcium solutions. To what extent such an inhibition of the pump may be present at normal calcium remains to be seen.

Conclusions

The results with low and high calcium support the concept that calcium contributes to overdrive suppression, and such contribution is likely to be greater for short drives. However, the demonstration that the largest overdrive-induced hyperpolarization is found in low calcium solution points to an electrogenic sodium extrusion as the major mechanism in overdrive suppression. This is further supported by the decrease in hyperpolarization and in pause duration when $[Na]^+$ is low and $[Ca]^+$ is presumably increased. The conclusion is in agreement with the fact that overdrive hyperpolarization is decreased or abolished under the following conditions which presumably impair the function of the sodium pump and increase cellular calcium: (1) cardiac steroids (Carpentier and Vassalle, 1971; Browning et al., 1979; Courtney and Sokolove, 1979), (2) the metabolic inhibitors dinitrophenol (Vassalle 1970), 2-deoxy-D-glucose (Carpentier and Vassalle, 1971), antimycin and sodium cyanide (Bhattacharyya and Vassalle, 1980), and (3) low temperature (Browning et al., 1979; Bhattacharyya and Vassalle, 1980). Furthermore, overdrive hyperpolarization is increased by a stimulation of the sodium pump (norepinephrine; Carpentier and Vassalle, 1971) or by a decrease in potassium conductance (Vassalle, 1970; Browning et al., 1979).

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References

Courtney KR, Sokolove PG (1979) Importance of electrogenic sodium pump in normal and overdriven sinoatrial pacemaker. Mol Cell Cardiol 11: 787-794
Diacono J (1979) Suggestive evidence for the activation of an electrogenic sodium pump in stimulated rat atria: Apparent discrepancy between the pump inhibition and the positive inotropic response induced by ouabain. J Mol Cell Cardiol 11: 5-30
Grossman A, Furchgott RF (1964b) The effects of frequency of stimulation and calcium concentration of $Ca^{2+}$ exchange and contractility in the isolated guinea-pig auricle. J Pharmacol Exp Ther 143: 120-130
Hogan PM, Spitzer KW (1975) Verapamil-induced increases in Purkinje fiber automaticity (Abstr). Fed Proc 34: 575
Ilsenberg G (1977a) Cardiac Purkinje fibres. $[Ca^{2+}]_c$ controls steady...
state potassium conductance. Pfluegers Arch 371: 71-76
Ishenberg G (1977) Cardiac Purkinje fibres. [Ca\(^{2+}\)]\(_i\) controls the potassium permeability via the conductance components \(g_{\kappa}\) and \(g_{N}\). Pfluegers Arch 371: 77-85
Reuter H (1965) Über die Wirkung von Adrenalin auf den cellulären Ca-Umsatz des Meerschweinchenvorhofs. Naunyn Schmiedebergs Arch Pharmacol 251: 401-412

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Skou JC (1975) The influence of some cations on an adenose triphosphatase from peripheral nerves. Biochim Biophys Acta 286: 394-401
Weidmann S (1955) Effects of cardiac ions and local anaesthetics on electrical properties of Purkinje fibers. J Physiol (Lond) 128: 568-582

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