The Importance of Calcium in Poststimulation Potentiation

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ABSTRACT Isotonic contractions recorded both before and during poststimulation potentiation in the toad isolated ventricle (Bufo marinus) revealed that the phenomenon of poststimulation potentiation was not altered by the presence or absence of the catechol amines, or by the specific amine antagonist, DCI. Similarly the inhibitors, sodium fluoride and sodium iodoacetate, were without effect. Changes in [Ca++] and [Na+] affected the degree of potentiation. High [Ca++] as well as the cardiac glycosides abolished it, low [Na+] and the absence of Mg++ depressed it. It has been shown that the percentage potentiation depends to some extent upon the total number of contractions occurring during the rapid stimulation phase. The amplitude of the contractions during this stage did not influence the degree of potentiation. These results are discussed in terms of Ca++ accumulation or redistribution associated with an early phase of the membrane depolarization.

The transient increase in cardiac contractility which follows a period of rapid stimulation (poststimulation potentiation) was initially reported by Langendorff (1885) following experiments in which he used isolated frog hearts. This augmentation has since been confirmed in many other species by numerous investigators. Rosin and Farah (1955) used rabbit auricular and ventricular strips, Furchgott and Sleator (1954) guinea pig auricles, Speirs (1959) rat ventricles, and Cattell and Gold (1955) cat capillary muscle.

Two major hypotheses have been formulated to explain the phenomenon. Rosin and Farah's results led them to postulate that both extrasystolic and poststimulation potentiation could be explained on the basis of a hypothetical potentiating substance released during the preceding contraction and partially utilized during the next. Whalen et al. (1958) extended this theory, suggesting that the potentiating substance released during contraction and destroyed during rest was either noradrenaline or a "noradrenaline-like substance." Earlier Ellis (1955) suggested that the substance might be hexosemonophosphate.
In contrast to this theory, involving the release and subsequent destruction of a potentiating substance, Cattell and Gold (1955) maintained that the spacing between the stimuli was the critical parameter involved in the augmentation and not the increased activity or the release of a substance during this activity.

The present investigation was carried out to further study poststimulation potentiation and in particular to determine whether or not this potentiation, as well as the classical "staircase" phenomenon (Nayler, 1960 a) could be explained in terms of Ca
t superscript + accumulation associated with rapid repetitive stimulation. Isolated toad ventricles were used throughout the study and the effect of changes in the perfusate Na
t superscript +, Ca
t superscript + and Mg
t superscript + concentrations, of drugs, and of metabolic inhibitors on the degree of potentiation was determined.

METHOD

Isolated ventricles from winter toads (Bufo marinus) were used in these experiments which were carried out at room temperature (19–22°C). Unless otherwise stated Ringer solution of the following composition was used:

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration</th>
</tr>
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<tbody>
<tr>
<td>NaCl</td>
<td>115 mM</td>
</tr>
</tbody>
</table>
| NaHCO
t subscripts 3 | 20.6 mM       |
| NaH
t subscripts 2PO
t subscripts 4 | 3.0 mM       |
| MgSO
t subscripts 7 | 1.2 mM        |
| Glucose            | 16.5 mM       |
| KCl                | 3.2 mM        |
| CaCl
t subscripts 2 | 0.55 mM      |

Oxygen was continuously bubbled through the perfusate. During dissection the pace-maker was destroyed by cauterization and the isolated ventricle stimulated directly with supramaximal rectangular pulses of 0.1 msec. duration delivered from a tektronix square wave generator. Ventricular isotonic contractions were recorded using the photoelectric apparatus previously described (Nayler and McCulloch, 1960).

After the dissection was completed a 30 min equilibration period was allowed, during which time the ventricle was stimulated at the basal rate of 6 beats per min. Following this equilibration period standard poststimulation potentiation was produced as follows:—The ventricle was stimulated at a rate of 6 beats per min. for 5 min., after which time the stimulation rate was raised to 46 beats per min. for 3 min. Following a 20 sec. pause, during which time stimulation was stopped, the basal rate of 6 beats per min. was resumed and the recording usually continued until the normal force of contraction was reestablished. The first poststimulation potentiation after the initial equilibration period was always discarded and the above schedule for the production of the standard potentiation rigorously adhered to.
1. Ions

In a series of experiments the percentage poststimulation potentiation was determined when perfusion fluids containing 0.325, 0.43, 0.65, 1.3, and 2.6 mM Ca ++ were used. In some experiments in which the perfusate contained 0.325 or 0.65 mM Ca ++, Mg ++ was deleted and the potentiation recorded compared with that in the same preparation when Mg ++ was present. Tonicity was not maintained.

In other experiments half the Na + of Ringer containing 0.325 mM Ca ++ was replaced by an osmotically equivalent amount of Li + (as lithium chloride), and potentiation produced as above.

2. Stimulation Rate

During the 3 min. of the rapid stimulation (46 beats per min.) which precede the pause and poststimulation potentiation, the interval between successive stimuli was adjusted in some experiments so that every second pulse arrived early during the diastole, but not during the absolute refractory period of the preceding beat. Thus the total number of stimuli arriving during the 3 min. period for any preparation was always 138 and in those experiments in which the stimulation rate remained isochronous, 138 contractions and relaxations all of equal magnitude were evoked. In those preparations in which every alternate stimulus arrived during the relative refractory period of the preceding beat every second beat was premature and consequently weak so that a series of 69 strong and 69 weak (incomplete) contractions was recorded.

In other preparations the frequency at which the ventricle developed its maximum contractile force was initially determined (i.e., peak of staircase), and this frequency then used throughout the period of rapid stimulation, the duration of which was adjusted so that the total number of contractions was always 138. In this way the number of stimuli which arrived during the rapid stimulation phase remained the same but the over-all activity differed from that in the standard preparation.

Additional experiments were carried out in which a variety of frequencies was used during the period of rapid stimulation, the duration of which was again adjusted so that a constant number of stimuli (138) was delivered to all preparations.

3. Drugs and Metabolic Inhibitors

The effect of noradrenaline1 (0.1 μg per ml) and of the specific catecholamine antagonist dichlor-isoproterenol2 (DCI 2 μg per ml) on the standard potentiation produced as described above was determined. Since other workers have shown that the naturally occurring amine stores are depleted by the prior injection of reserpine3 (Krayer and Paasonen, 1957) or of syrosingopine4 (Orlans et al., 1960), potentiation was studied

1 Noradrenaline as levophed, Winthrop Laboratory product.
2 Dichlorisoproterenol (DCI), Eli Lilly product.
3 Reserpine, as serpasil, Ciba product.
4 Syrosingopine, Su 3118, Ciba product.
in ventricles removed from toads which had been treated with one or the other of these drugs on 3 consecutive days before use.

In other preparations the effect on potentiation of strophanthin-G\(^4\) (100 \(\mu\)g per ml), lanatoside C\(^6\) (100 \(\mu\)g per ml), quinidine sulfate\(^7\) (100 \(\mu\)g per ml), and of the metabolic inhibitors dinitrophenol (DNP), sodium iodoacetate, and sodium fluoride was determined. Earlier investigations carried out in this laboratory indicated that natural hibernation greatly reduced the sensitivity of the isolated toad heart to the glycosides and these seemingly high concentrations were necessary in order to produce a positive inotropic response when using hearts isolated from winter toads (Nayler, 1957a).

\[\text{Fig. 1. Standard poststimulation potentiation. } b, \text{ basal rate 6 beats per min. } f, \text{ fast rate 46 beats per min. for 3 min. } p, \text{ pause (20 sec.). Note potentiation following return to basal rate of stimulation after the pause. Read from left to right.}\]

**RESULTS**

In Fig. 1 the typical poststimulation potentiation pattern recorded during these experiments is displayed. It can be seen that, following the 20 sec. pause, an increased force of contraction which slowly declined to the pre-stimulation level was recorded. This decline was always hyperbolic. In some experiments the duration of the pause was varied between 10 sec. and 10 min. The potentiations thus recorded varied according to the length of this rest interval so that the greatest potentiation was recorded when the pause was short (10 sec.), and the percentage potentiation decreased exponentially as the duration of the pause increased. No potentiation occurred in these experiments when the duration of the pause exceeded 7 min.

Poststimulation potentiation and the rate of decay of the potentia-

\(^4\) Strophanthin-G: ouabain, Arnaud laboratorie nativelle product.
\(^6\) Lanatoside-C, cedilanide, Sandoz Ltd. product.
\(^7\) Quinidine sulfate as quinicardine, Laboratorie nativelle product.
tion showed a marked dependence upon the calcium concentration, $[\text{Ca}^{++}]_s$, of the perfusate, the greatest potentiation and the most rapid decay rates being recorded when the $[\text{Ca}^{++}]_s$ was low. The results of a typical single preparation are displayed in Fig. 2 where the poststimulation potentiation recorded after the 20 sec. pause when the perfusate $[\text{Ca}^{++}]_s$ was either 0.325, 0.43, 1.3, or 2.6 mM is shown.

In Fig. 3 are summarized the results of four other preparations in which the perfusate $[\text{Ca}^{++}]_s$ was varied, the potentiation being expressed as the percentage change relative to the force of contraction recorded during the initial basal rate stimulation period. Reduction of the rest pause from 90 to 10 sec. failed to modify the potenitations recorded when the $[\text{Ca}^{++}]_s$ was either 1.3 or 2.6 mM, making it unlikely that the smaller potenitations recorded

\[ [\text{ }] \text{denotes concentration.} \]
when the perfusates used contained elevated [Ca++] were, in fact, due to increased rates of decay.

Deletion of Mg++ from the perfusate depressed potentiation, the depression being most marked when a low [Ca++] Ringer was used. The results of two such experiments in which Mg++ was deleted from Ringer containing 0.325 mM Ca++ are shown in Fig. 4. Similarly substitution of half the Na+ by Li+

decreased the potentiation recorded following the standard rapid rate stimulation. In Fig. 5 the results of four Li+ substitution experiments are summarized, the [Ca++] being 0.325 mM in all cases. The deletion of Mg++ from 50 per cent Na+ 50 per cent Li+ Ringer resulted in further depression of the potentiation.

**Stimulation Rate**

When the interval between successive stimuli during the rapid stimulation phase was adjusted so that every second impulse arrived soon after the re-
Figure 4. Poststimulation potentiation recorded in two preparations. A, in the presence of, and B, in the absence of Mg++. In each case only a single beat of the initial basal rate phase has been shown and the recorder was run at fast speed to separate this from the first beat of the rapid stimulation period. p, pause. Read from left to right. The absence of Mg++ depressed potentiation.

Figure 5. Poststimulation potentiation recorded in four preparations in which Ringer containing 100 per cent Na+ or 50 per cent Na+ 50 per cent Li+ was used. Potentiation is expressed as the percentage change in amplitude of isotonic contraction relative to the amplitude recorded during the initial basal rate stimulation.

Fractory period of the preceding beat, then half the contractions were premature and weak. Under these conditions the potentiation was the same as that recorded when each stimulus gave rise to a fully developed contraction. The results of two such experiments are shown in Fig. 6. In Fig. 6 A all stimuli
Figure 6. Poststimulation potentiation recorded in two preparations. A, 3 min. rapid stimulation associated with 138 contractions all of equal amplitudes. B, 3 min. rapid stimulation associated with 69 weak and 69 strong contractions. In the upper tracing the recorder was run at fast speed to separate the last basal rate beat from the first rapid rate one. p, pause of 20 sec. Read from left to right.

<table>
<thead>
<tr>
<th>No.</th>
<th>Basal rate</th>
<th>Fast rate</th>
<th>No. of stimuli</th>
<th>Potentiation (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>6/min.</td>
<td>60/min.</td>
<td>180</td>
<td>1070</td>
</tr>
<tr>
<td></td>
<td>6/min.</td>
<td>46/min.</td>
<td>180</td>
<td>1100</td>
</tr>
<tr>
<td>2.</td>
<td>6/min.</td>
<td>60/min.</td>
<td>180</td>
<td>350</td>
</tr>
<tr>
<td></td>
<td>6/min.</td>
<td>46/min.</td>
<td>180</td>
<td>342</td>
</tr>
</tbody>
</table>

initiated contractions of equal amplitude, whereas in Fig. 6 B every second stimulus caused only a small contraction superimposed upon the first. However, the degree and rate of decay of potentiation recorded are the same in both instances.

In those preparations in which the peak staircase frequency was used and the duration of the rapid rate phase adjusted so that 138 stimuli were de-
Poststimulation potentiation recorded both before (pp) and after (pp') the administration of dichlorisoproterenol (DCI 2 μg per ml). At this concentration DCI inhibited the inotropic activity of 0.1 μg per ml noradrenaline (NA). Poststimulation potentiation was not affected. Read from left to right.

Figure 7.

Poststimulation recorded before (A) and 30 min. after (B) the addition of strophanthin-G (100 μg per ml). In both instances only part of the initial basal rate stimulation period was recorded and the recorder was run at fast speed to separate the last beat of the basal rate stimulation from the first beat of the rapid rate stage. p, 20 sec. pause. Read from left to right.

Figure 8.

delivered, the percentage potentiation remained the same as that in which 3 min. stimulation at 46 beats per min had occurred; i.e., the increased mechanical activity associated with peak frequency failed to modify the resultant potentiation.

This relationship between the number of stimuli received and the degree
of potentiation was further displayed in those experiments in which the frequency throughout the rapid rate stimulation phase was varied but the total number of stimuli kept constant. The results of two such experiments are shown in Table I, with two stimulation frequencies used in both preparations.

![Graph comparing the time taken by strophanthin-G (100 μg per ml) to develop inotropic activity with the time taken to abolish poststimulation potentiation. Inotropic activity was measured in terms of the total useful work done by the isolated winter toad heart. Strophanthin-G added at the arrow.](image)

In some experiments the duration of the fast stimulation phase was not adjusted so that different numbers of stimuli arrived during the fast stimulation periods. Under these conditions the percentage potentiations varied according to the total number of stimuli received.

**Drugs and Metabolic Inhibitors**

Normal potentiation was recorded in the presence of noradrenaline (0.1 μg per ml) and in those experiments using ventricles removed from toads
whose natural amine stores had been depleted either by reserpine or by syrosingopine. DCI, when administered in sufficient concentrations to abolish the positive inotropic activity of 0.1 µg per ml noradrenaline, failed to modify the poststimulation potentiation pattern (Fig. 7). DNP abolished the potentiation only when the ventricle was severely depressed.

In marked contrast to these results recorded in the presence of noradrenaline, DCI, and the metabolic inhibitors, the cardiac glycosides profoundly modified poststimulation potentiation as produced in this study. Thus strophanthin-G and lanatoside-C, when given in doses sufficiently high to produce a positive inotropic response in the isolated winter toad heart (Nayler, 1957), both progressively depressed and finally abolished the potentiation (see Fig. 8). In Fig. 9 the time course of the glycoside (strophanthin-G) depression and ultimate abolition of potentiation has been plotted together with the time taken by the drug to develop positive inotropic activity, when given to the isolated heart. There appears to be some correlation between the rate of development of the positive inotropic response (Nayler, 1957 a) and the depression of poststimulation potentiation.

**DISCUSSION**

In these experiments rapid stimulation of the isolated toad ventricles for short periods resulted in potentiation of the ensuing basal rate contractions. The potentiation was maintained throughout a rest period of at least 20 sec. and the degree of potentiation was dependent, to some extent, on the frequency with which contractions occurred during the rapid stimulation phase and not on the amplitudes of the individual contractions. High [Ca++] and low [Na+] depressed potentiation, low [Ca++] enhanced it.

In general the results reported here agree with those published recently by Spiers (1959) who found that raised [Ca++] depressed and low [Ca++] enhanced the potentiation he recorded in rat ventricle strips. Also in agreement with Spiers, and as would be predicted from the results of Niedergerke (1956, 1957, 1959) if Ca++ is involved in the phenomenon, replacement of half of the Na+ by an osmotically equivalent amount of Li+ depressed potentiation. In the present study some antagonism was found between Mg++ and Ca++ so that in low [Ca++] Ringer deletion of Mg++ depressed potentiation. However, deletion of Mg++ from high Ca++ Ringer was without any appreciable effect. It is difficult to understand why, in their study using cat isolated papillary muscle, Penna and Garb (1956) failed to detect any change in extrasystolic potentiation following variations in the [Ca++] and [Mg++] of their perfusate. Their recordings were, however, isometric whereas those of the present study were isotonic. Recently Shanes and Bianchi (1960)
have demonstrated a difference in Ca++ flux between isotonic and isometric skeletal preparations and a similar difference in cardiac muscle may explain the above discrepancy.

Isotonic rather than isometric preparations were used in this study since other experiments have shown that physiological events which are believed to involve surface-acting mechanisms are more pronounced under isotonic than under isometric conditions (Nayler 1960 b). It is possible that the movements of the myofibrils during isotonic contractions contribute to the displacement of fluids throughout the membrane-lined interspaces of the cardiac cells, thus allowing the exposure of a larger surface area to the changed extracellular environment than would occur under isometric conditions.

If, as Rosin and Farah (1955) postulated, potentiation is due to the release of a potentiating substance, then this substance must be released in response to stimulation and not to the actual beat associated with the stimulus. The results reported here contrast with those of Whalen et al. (1958), since the presence or absence of noradrenaline failed to modify the potentiation and the specific antagonist DCl, was without effect. A similar lack of correlation between noradrenaline activity and potentiation recorded in the classical "staircase" has already been described (Nayler, 1960 a).

As the result of their studies Cattell and Gold (1955) concluded that "the factor responsible for the increased force of contraction during treppe and at higher frequencies of stimulation or following a single extra stimulus is the rhythm or spacing between stimuli rather than the increased activity per se." Such a conclusion has been confirmed in the present study, the results of which make it unlikely that the release of any substance as the result of contraction or relaxation is involved in the potentiation. The potentiation is, however, closely related to the frequency of the stimulus and to the interval between contractions. This link between the frequency of stimulation and the magnitude of contraction supports the studies of Vaughan Williams (1959) in which he used rabbit atra.

Cattell and Gold (1955) reported that a single extra stimulus, when applied to a regular slow frequency series of isometric contractions by the cat papillary muscle preparation, resulted in maximum potentiation of the following beat or beats. In an earlier communication (Nayler, 1960 a) it was reported that such a potentiation does not follow the introduction of a single extra stimulus into a series of slow regular contractions of ventricular strips removed from winter toads. During the present study it was further observed that such a single extra stimulus failed to potentiate the following beat or beats of the isotonically contracting toad ventricle. This difference between the response of the cat papillary preparation and the ventricular muscle of the winter toad to a single extra stimulus suggests that a species difference in the ionic exchange associated with each stimulus and contraction may exist.
In the toad ventricular preparation more than one extra stimulus appears to be necessary to produce potentiation of the following contractions. Digitalis and high [Ca++] abolished, and low [Na+] depressed the augmentation. The importance of Ca++ in cardiac activity is well documented. Luttgau and Niedergerke (1958) suggested that accumulation of Ca++ in or about the vicinity of the membrane regulated cardiac contractile force. More recently Bianchi and Shanes (1959) postulated that increased Ca++ entry is associated with depolarization of the membrane and Holland and Sekul (1959) noted increased Ca++ influx in isolated rabbit atria following the administration of ouabain.

It is possible to explain poststimulation potentiation and the action of the cardiac glycosides of high and low [Ca++] and of reduced [Na+] on this potentiation if it is assumed that Ca++ entry is associated with an early stage of contraction—probably the depolarization stage. Ca++ influx is then related only to the number of stimuli received and not to the resultant mechanical events.

Under these circumstances the degree of potentiation would vary according to the external [Ca++], the number of stimuli received within a given time and the presence or absence of other substances which affect the rate of Ca++ flux. The similar time course followed in the development of inotropic activity and the abolition of poststimulation potentiation by the glycosides strophanthin and lanatoside-C suggests that these two phenomena may be related and that a common site of action might be involved in both events. This site could be that which regulates the distribution of Ca++ within the cardiac cell and across the membrane.

This similarity between the response of the isolated hearts to Ca++, the glycosides, and to rapid stimulation is also evident in the activity of the metabolic inhibitors. Thus neither sodium fluoride nor sodium iodoacetate interferes with the positive inotropic activity of the glycosides or of Ca++, and it has been shown above that they do not abolish poststimulation potentiation. Severe depression of the myocardium by DNP, however, abolishes inotropic activity of the glycosides and of Ca++ (Ellis, 1953; Nayler 1957 b) and, as was seen above, abolishes poststimulation potentiation.

In conclusion then, it seems possible that poststimulation potentiation as well as the classical "staircase" phenomenon (Nayler 1960 a) may be due to the accumulation or redistribution of Ca++ associated with rapid repetitive stimulation. Provided that the frequency during this rapid rate stimulation is sufficiently high and the pause separating the last rapid frequency beat from the first poststimulation beat is not too long, then the beats immediately following the pause will be enhanced, the percentage potentiation being proportional to the Ca++ accumulation or redistribution associated with the previous rapid stimulation. Following the return to the basal stimulation fre-
frequency more time will occur during diastole and rest for the Ca++ accumulated at specific sites during the rapid stimulation to be lost. This loss is reflected in the exponential decay curves of the potentiations. The fact that the glycosides as well as Ca++ abolish this potentiation strengthens the theory that these drugs alter myocardial contractility via a Ca++-dependent mechanism.

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