MULTIPLE FIRING OF FROG'S VENTRICLE IN RESPONSE TO A SINGLE STIMULUS IN RINGER'S SOLUTION WITH EXCESS CALCIUM

Yasu-Ichiro Fukuda

Department of Physiology, Chiba University School of Medicine, Chiba

In the author's previous reports it was shown that the well documented "calcium rigor" in the ventricle of the frog's heart preparation perfused with Ringer's solution containing excess CaCl₂ is a "calcium tetanus" evoked by multiple discharges in response to an excitation conducted from the auricle which beats normally. The "calcium tetanus" subsided when A-V block appeared. In an attempt to obtain further evidences to support this postulate, present experiments were conducted to ascertain whether or not a ventricular preparation of frog's heart will respond with multiple firings to a single direct electrical stimulus, when the Ca++ concentration of the bathing Ringer's solution is raised. This was actually found to be the case, and various conditions which inhibit the occurrence of the multiple firings were also clarified.

METHODS

Ventricular preparations of Bufo vulgaris, after removing the A-V ring muscle, were perfused by Straub's cannula with Ringer's solution well oxygenated with pure O₂. A direct maximal stimulus (a square wave pulse of 2.5 msec duration) was given at the base of the preparation with a pair of silver electrodes. The electrogram was obtained by a pair of cotton threads placed at the midde of the ventricle. The movements of a weight plate (0.5 g) attached by a thread to the apex of the ventricle were recorded photoelectrically as the mechanogram.

The composition of the Ringer's solution and the method of analysis of the ventricular Ca content were the same with that described in the preceding report. With the Ringer's solution adjusted at pH 7.0-7.2 by a NaHCO₃/NaH₂PO₄ (30/1) buffer, no precipitation appeared even with high Ca++ concentration (10 mM) or with high Mn++ concentration (8 mM).

RESULTS

Multiple firing in frog's ventricle in response to a single direct stimulus. As shown in Fig. 1, extra Ca++ first increases and then, in large doses, depresses
MULTIPLE FIRING IN FROG'S VENTRICLE

the mechanical response to a direct stimulus. Together with this depression, the electrical response becomes small in size and shortened in duration. When the ventricular response thus tends to become a small localized one, an abrupt firing of electrical responses begins to appear after the initial response. With repetition of stimulation, a full-grown, long continued ventricular firing accompanying a long continued ventricular contraction appears. The synchronized repetitive firing starts with high frequencies (above 500 c/min) and stops suddenly, when the frequency reduces to about 200 c/min. With appropriate time intervals this firing could be repeatedly induced. When a stimulus was given shortly after cessation of the firing, only a small

![Graph](image)

**Fig. 1.** Multiple firing in response to a single stimulus in the presence of excess Ca++. From above, ST: time of stimulation, MG: mechanogram, EG: electrogram. A downward deflexion just preceding each electrogram is a stimulus artifact. \(n\times\text{Ca}\) means \(n\)-times higher Ca concentration than the normal value in Ringer's solution. These are the same in the following figures.

![Graph](image)

**Fig. 2.** Course of development of multiple firing in response to a single stimulus after cessation of the preceding firing.
electrical response with a localized contraction not perceptible in the mechano-
gram appeared (Fig. 2). As is noticed in this figure, a visible contraction of
the ventricle appears only when the multiple firing begins to reappear. There
is a marked delay in the appearance of contraction due to the multiple firing
after the initial local electrical response. The multiple firing seemed to start
at the base of localization of the initial response to an electrical stimulus.
This starting mechanism became temporarily blocked after spontaneous ces-
sation of the long continued firing. Concerning the mechanism of cessation
of the firing, discussion will be made later. With these in mind, various
factors which inhibit the multiple firing were examined as follows.

Factors which inhibit the multiple firing. The above described multiple firing
in response to a single stimulus was easily induced at temperatures above
20°C. However, at temperatures lower than this the firing became difficult
to be induced, just as is the case with a whole heart preparation automatically
driven. Fig. 3 shows the results obtained at a temperature of 10°C. As seen
in the figure, even with excess Ca++, the above mentioned suppression of
contraction cannot be observed. In the electrogram, the initial response
remains unaltered and the shortening of the response duration is less marked
than at high temperatures. Thus the absence of long continued firing seemed
to be due to the failure of excess Ca++ to localize the initial response at low
temperatures.

![Graph showing inhibition of multiple firing due to excess Ca++ at a low temperature.](image)

Fig. 3. Inhibition of multiple firing due to excess Ca++ at a low temperature.

The effect of excess Ca++ to localize the excitation and contraction was
found to be inhibited even at high temperatures when the K+ concentration
was raised to 2.5 times the normal (Fig. 4). In this case the start of multiple
firing was also inhibited. Thereby the relaxation of contraction became
retarded with increasing Ca++ concentration and the base line was progres-
sively elevated.
Epinephrine was unique in that it counteracted the effect of excess Ca++ and rather increased both the duration of contraction and electrical response even in the presence of high Ca++ concentration (Fig. 5). Thus no repetitive firing could be started under epinephrine. Moreover, the multiple firing once started by excess Ca++ could be suppressed by epinephrine and a normal response to each direct stimulus reappeared.

*Factors which inhibit the start of the multiple firing from the localized initial response.* Procaine ($5\times10^{-6} \text{ g/ml}$) and tetrodotoxin ($5\times10^{-10} \text{ g/ml}$) were found to inhibit the multiple firing to a single stimulus in the presence of excess Ca++. As is noticed in Fig. 6 and 7, these drugs in concentrations which do not materially influence the response to a single stimulus in normal Ringer's solution, often induce a short continued firing of low frequencies at Ca++
Fig. 6. Effect of procaine on multiple firing due to excess Ca++. Procaine: 5 x 10^-6 g/ml.

Fig. 7. Effect of tetrodotoxin on multiple firing due to excess Ca++. Tetrodotoxin: 5 x 10^-10 g/ml.

concentration of 4-6 times the normal. These firings are accompanied by undulating contractions; quite resembling ventricular flutter in mammalian hearts. However, further elevation of the Ca++ concentration did not induce the multiple firing, in spite of complete localization of the initial response. Thus the mode of inhibition of the multiple firing by these drugs was quite different from that by low temperatures, excess K+ and epinephrine as above mentioned.

Procaine and tetrodotoxin are known to inhibit the action potential carried by Na+. In this respect, it was quite remarkable that Mn++, which is said to inhibit action potential carried by Ca++, did not inhibit the multiple firing due to excess Ca++, even at high concentration (8 mM). As shown in Fig. 8, because of the suppression of contraction induced by excess Mn++ in normal Ringer's solution, the necessary condition for starting the multiple firing due
to excess Ca++ mentioned above can be noticed only in the electrogram. With the start of the multiple firing a pronounced continued contraction can appear. This might suggest that “Ca entrance” effectively prevented by Mn++ in a single response would be markedly facilitated by the multiple firing.

Relationship of the increase in the Ca content of the ventricle to the multiple firing. It has previously been reported that when “calcium tetanus” of the ventricle is induced in a whole heart preparation an increase in the Ca content of the ventricle (a net gain in muscle tissue) is observed and this does not occur when “calcium tetanus” is prevented at low temperatures or by excess K+. However, whether the net gain of Ca is the cause of the multiple firing or its result could not be determined in spontaneously beating hearts. Therefore, the relationship of the increase in the Ca content of the ventricular muscle tissue to the multiple firing was examined as follows.

The Ca content of the ventricular muscle perfused with Ringer’s solution containing excess Ca++ was compared with that perfused by normal Ringer’s solution. As shown in Fig. 9 there is no definite increase in the Ca content when the ventricle remains unstimulated in Ringer’s solution containing excess Ca++. A marked increase is detected only when the ventricle is stimulated and the multiple firing together with “calcium tetanus” appears. However, even when stimulation is given repeatedly the increase is dismissed, if the application of epinephrine inhibits the onset of the multiple firing. Thus it seemed plausible that the increase in the ventricular Ca content is the result of the appearance of the multiple firing. That “Ca entrance” will be facilitated during the firing period might be in accord with the postulation above mentioned, i.e. “Ca entrance” effectively prevented by Mn++ in a single response will be facilitated by the multiple firing. It may be here added that when the NaCl in Ringer’s solution is partially replaced with isotonic
FIG. 9. Ca content of frog's ventricles in various conditions. Each column represents the mean value with S.E. Stimulation was applied at a rate of 12/min. Experimental temperatures: 23-25°C.

FIG. 10. Spontaneous contracture in frog's ventricle due to excess Ca++ enhanced by low NaCl concentration. Note a single response to each stimulus during contracture.

sucrose solution an increase in the ventricular Ca content spontaneously occurs (FIG. 9) and with this a "calcium contracture" without accompanying electrical response is set off (FIG. 10).

DISCUSSION

It has been shown that the frog's ventricle responds to a single direct stimulus with the multiple firing accompanied by a long sustained contraction, when the Ca++ concentration of the bathing Ringer's solution is raised to 8 times the normal value. Thus the previous postulate that the appearance of "calcium tetanus" of the ventricle in the whole heart preparation is due to
multiple firing of the ventricle in response to a supraventricular excitation conducted to the ventricle has been substantiated. It was also shown that the multiple firing due to excess Ca++ appears only under conditions in which the electrical response will be localized, while the excitation may spread out through the whole ventricle. This spread of excitation was found to be inhibited by procaine or tetrodotoxin, while the localization of the response was inhibited by epinephrine, excess K+ and by low temperatures. All these conditions inhibited the induction of the multiple firing due to excess Ca++. The mechanism of the spread of excitation from the localized response is difficult to define. However, the resulting multiple firing would indicate an appearance of excitation in other parts of ventricular tissue just as is the case of ventricular flutter in the mammalian heart. Actually ventricular flutter was observed when the frequency of multiple firing became reduced. The multiple firing of the ventricle thus seemed to be flutter waves of high frequencies. Ca++ and local anesthetics have been said to ‘stabilize’ excitable membranes. The underlying mechanisms have been analyzed by WEIDMANN on Purkinje fibers. It has been shown that while local anesthetics lower considerably the rate of rise and ‘overshoot’ of the action potential, a 4-fold decrease or 4-fold increase of the Ca++ concentration in the extracellular fluid has no marked effect on the size and shape of the action potential. However, more depolarization is required to excite a fiber in Ca-rich solutions, and less depolarization in Ca-poor solution. This accounts for the ‘stabilizing’ effect of Ca++, which differs from that of local anesthetics. Tetrodotoxin has also been shown to have a similar effect to a local anesthetic (procaine) in suppressing ‘Na spike’ of the ventricular muscle fiber of frog’s heart. Thus the special circumstances that Ca-rich solution alone can start the multiple firing, while local anesthetics and tetrodotoxin prevent it would be understandable.

Concerning the mechanism of prevention of localization of the response due to excess Ca++ by K+, epinephrine and low temperature, following comments may be mentioned. That K+ antagonize the effect of Ca++ can be easily understood by the evidence presented by HOFFMAN and SUCKLING. However, explanation of the antagonism of epinephrine against the effect of excess Ca++ would be complicated. It is known that epinephrine lowers the threshold in Purkinje fibers, which is just opposite to the effect of excess Ca++. Moreover, epinephrine prolongs the action potential duration of the frog’s ventricle while excess Ca++ shorten it. Actually the shortening of the duration of electrical response by excess Ca++ was completely inhibited by epinephrine (Fig. 5). In this respect, it was quite remarkable that the localization of the response due to excess Ca++ was antagonized by K+ without influencing the Ca++ effect upon electrical response duration. Effect of low temperature upon the effect of excess Ca++ resembles to that of epinephrine.
in that the shortening of the duration of electrical response was difficult to occur. These circumstances deserve further elucidation.

The fact that the net gain in the Ca content of the ventricle is the result of the multiple firing and not the cause of it, would aid in elucidating the mechanism of "calcium tetanus". Spontaneous cessation of the multiple firing after reduction in the frequency of the firing might be the result of an excess accumulation of Ca in the myocardium during the period of multiple firing. Loss of the gained Ca during succeeding silent period would give chance again to induce a multiple firing in response to a single stimulus. Thus the mechanism of multiple firing seemed to be enhanced by a moderate increase, but to be inhibited by an excess increase in the Ca content of the ventricle. The cellular Ca seemed to influence the blocking effect of excess Ca++ in the medium. In a preceding report, it was suggested that a net gain of Ca content of the ventricle might be the cause of the multiple firing. This concept has been revised by the new evidences presented in the present report.

SUMMARY

It was shown that frog's ventricle respond with multiple firing accompanied by a long continued contraction in response to a single direct electrical stimulus when the concentration of Ca++ in the bathing Ringer's solution is raised up to about 8 times the normal concentration. This was shown to be due to the effect of excess Ca++ to localize the response by partial blocking, while a possibility of spreading the excitation to the whole ventricle still remains. The localization of the response was prevented by excess K+, epinephrine and by low temperatures. The spread of excitation was completely blocked by procaine or tetrodotoxin. All these conditions were found to inhibit the multiple firing accompanying a long sustained contraction, which is estimated due to an increase of Ca content in the ventricular tissue (a net gain). The net gain in the ventricular Ca content was found to be due to the result of multiple firing of the ventricle.

The author is deeply indebted to Prof. Tokuro Fukuda for his helpful advice and criticism throughout this investigation and for preparing the manuscript.

REFERENCES


