Summation and Tetanus in Cardiac Muscle. Effects of Temperature, Epinephrine and Digitoxin.* (20851)

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It is generally recognized that fundamental understanding of the mechanisms of cardiac weakness and failure must rest upon knowledge of the contractile properties of cardiac muscle. Information regarding differences and similarities between the characteristics of cardiac and skeletal muscle is of importance in this regard. While it is usually stated that cardiac muscle does not exhibit the phenomena of summation and tetanus, reports in both the older(1-3) and more recent(4,5) literature indicate that such responses are at least potentially possible. It is the purpose of this report to confirm the occurrence of summation and incomplete tetanus in the isolated cardiac tissue of the frog and rat under stated conditions and to present data on the mechanism of its production and its modification by certain cardioactive substances in the case of the frog.

Methods. Ventricular strips were prepared from the mid-portion of the ventricle of *Rana pipiens*, and suspended in oxygenated Ringer's solution. The base of the muscle was held in a specially designed clamp into which were incorporated a pair of chlorided silver electrodes. The upper end of the strip was connected to a Statham strain gauge (Model 67-0.3-800) whose output was amplified and recorded on a Grass ink-writing oscillograph. Contractions were essentially isometric and were recorded with the muscle under an initial tension of 500 mg. Stimuli were applied from a square wave stimulator either directly to the base of the muscle through both clamp electrodes or with one of the clamp electrodes serving as cathode and the bath serving as anode. Prior to use the animals were kept in running water at temperatures of 18-22°C. The experiments were performed during the period from February to June. Right ventricular strips were prepared from albino rats of the Holtzman-Rolfsmeyer strain after the method of Feigen *et al.*(6). Animals were killed by a blow on the head and the strips suspended in buffered medium(6) within 1-2 minutes of the time of death. The bath was gassed with 95% O_2-5%CO_2 and maintained at a temperature of 38°C. Suspension, stimulation and recording were the same as already described for the frog. In both frog and rat experiments strips were driven at a basic rate of one beat per second and were allowed to equilibrate for an initial period of one-half hour before experimental observations were begun. Details of experimental procedures will be presented with results.

Results. The classical response of frog cardiac muscle to repetitive stimulation is apparent in Part A of Fig. 1. At a temperature of 22°C an increase in the rate of stimulation from one pulse per second to 5 pulses per second resulted in a decrease in the magnitude of the individual twitches, and a reduction in the total tension developed by the strip during the period of rapid stimulation. More rapid rates of stimulation produced similar responses. There is no evidence of summation of individual contractions. If the temperature of the bath is raised to 30°C, however, the response pictured in Part B of the record is obtained. Stimulation at a rate of 5 pulses per second now results in a reduction in the magnitude of the individual contractions, but the diastolic baseline rises and

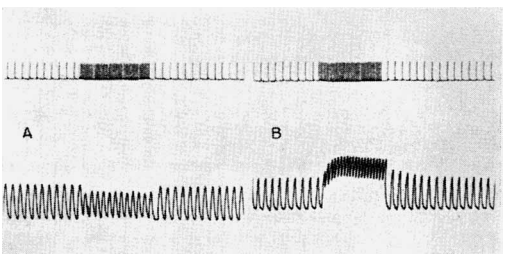

FIG. 1. Production of incomplete tetanus in frog ventricle strip by elevation of temperature. Stimulus record above, myogram below.
A—temp. 22°C B—temp. 30°C

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the total tension developed by the strip during the period of rapid stimulation exceeds that produced at the slower rate of stimulation. Summation of contractions is apparent and the record is comparable to that of incomplete tetanus in skeletal muscle. Upon cessation of rapid stimulation the baseline returns to its previous level and the magnitude of the individual beats likewise returns to pre-tetanic levels. It is of interest to note that the first few beats following cessation of rapid stimulation are, however, of increased amplitude. Experiments on 30 preparations indicated that the phenomenon could be consistently reproduced, the temperature necessary for its production varying from 26°C to 34°C with an average of about 30°C. Frequencies of 5 to 10 per second were optimal for its production. These results clearly indicate that summation and incomplete tetanus in frog cardiac muscle strips can be regularly produced by elevation of bath temperature.

Since the phenomena of summation and tetanus would seem to be related to the well known occurrence of treppe in cardiac muscle it was of interest to study the effect of various cardioactive substances on the phenomenon in the light of the recent reports of Hajdu and Szent-Györgi(7,8). Substitution of serum for Ringer's solution in the bath was without effect, and reduction in K ion concentration produced inconsistent results.

Addition of epinephrine hydrochloride (Parke-Davis) to the bath in concentration of 10^{-6}, consistently abolished the tendency for cardiac strips to summate at high temperatures. The records of such an experiment are reproduced in Fig. 2. Part A shows the incomplete tetanus produced by stimulation at the rate of 5 per second at 32°C and the almost completely fused response to 10 stimuli per second at this temperature. Addition of epinephrine to the bath one minute prior to the record of Part B resulted in abolition of the summated response. There is a slight elevation of diastolic baseline but there is no summation of beats during the period of rapid stimulation and the tension developed during this period is less than that developed at a driving rate of one per second. Part C indicates the results of tetanic stimulation 5 minutes after the administration of epinephrine. Although the positive inotropic action of the drug has markedly diminished, there is no summated response to stimuli at 5 or 10 per second. Following washing out of the drug with Ringer's solution the summated response could be once more obtained (not shown in this figure).

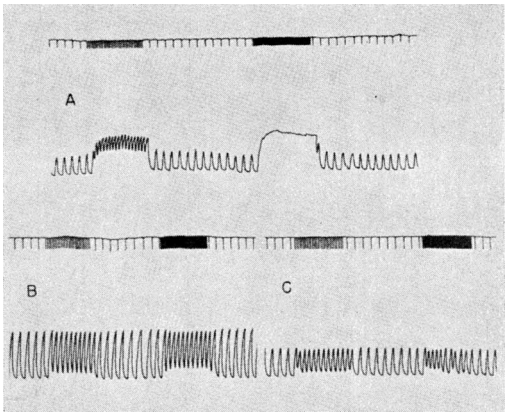

FIG. 2. Inhibition of summated response by epinephrine. Temp. 32°C. Stimulus record above, myogram below.
Part A—Stimulation at 5 and 10/sec.
B—One min. after addition of 10^{-6} epinephrine to bath.
C—Five min. after addition of epinephrine.

Hajdu and Szent-Györgi(8) reported that digitalis glycosides abolished the treppe response in frog heart and suggested that action of these substances on the permeability of the muscle membrane to K ions was responsible for their effects. We accordingly investigated the influence of digitoxin (Digitoxin, Abbott) in concentration of 0.3 μg per ml on the development of summation and tetanus in frog ventricle strips. Observations at temperatures of 30°C and above suggested that the glycoside did not abolish, but actually favored the development of the summated response. This effect was clearly demonstrated by the development of summation and tetanus in digitalized strips at temperatures at which untreated strips failed to show these responses. The records of such an experiment conducted at 22°C are reproduced in Fig. 3. Within 5 minutes of the administration of the drug the unsummated response to repetitive stimulation at 5 beats per second was converted to an incomplete tetanus, and after 15 minutes the

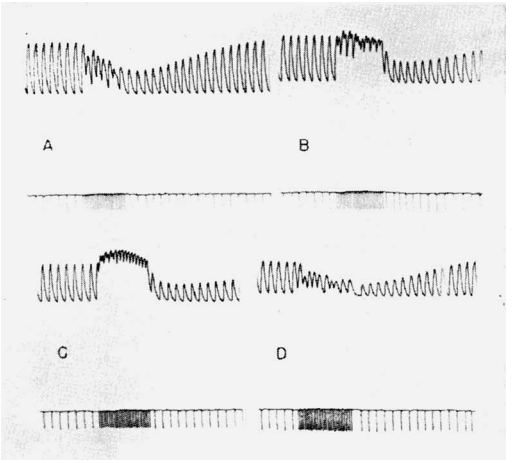

FIG. 3. Production of summated response by digitoxin. Temp. 22°C. Myogram above, stimulus record below.
A—Untreated strip.
B— 5 min. after digitoxin, 0.3 μg/ml.
C—15 min. after digitoxin.
D—30 min. after washing with Ringer's.

response was well established. Washing with Ringers gradually removed the effects of the drug and the muscle responded in the untreated fashion. Re-digitalization resulted in a return of the summated response.

It was of interest to determine whether the inhibitory action of epinephrine on summation and tetanus at higher temperatures also extended to that produced as a result of digitalization. Addition of epinephrine to digitalized strips consistently abolished the summated response.

In an approach to an understanding of the observations described, modification of the duration of the refractory period of the muscle strips offered a possible explanation. Measurements were accordingly made, the refractory period as here defined being the minimal time interval between the driving stimulus at a rate of one pulse per second and a supramaximal test shock of 14 milliamps magnitude, necessary to produce a detectable mechanical response. In all cases, development of summated responses at elevated temperatures was associated with shortening of the refractory period and abolition of these responses by cooling was accompanied by lengthening of refractoriness. Typical changes in refractory period induced by digitoxin and epinephrine are graphically summarized in Fig. 4. It can be seen that the development of summation as the result of digitoxin action is associated with reduced refractoriness and the inhibitory action of epinephrine on this phenomenon with increased duration of the refractory period. These findings are in agreement with classical teaching that the failure of cardiac muscle to summate under usual conditions is a function of the length of its refractory period, and with reports in the older literature(1,2) in which cardiac tetanus was produced by procedures which shortened the refractory period. The reduction in duration of the refractory period noted with digitoxin is in agreement with the observations of Macleod(9) and with the recent report of Woodbury and Hecht(10) on the effects of cardiac glycosides on single ventricular fibers. The contrary action of epinephrine is compatible with reports indicating slowing of repolarization in cardiac muscle under the influence of this agent(11,12), and the associated inhibition of the summated response confirms the findings of Spadolini(13) in the case of toad ventricle. It is of interest that these 2 potent cardio-active substances, each capable of increasing the strength of cardiac contraction, should show antagonistic properties in relation to the duration of its

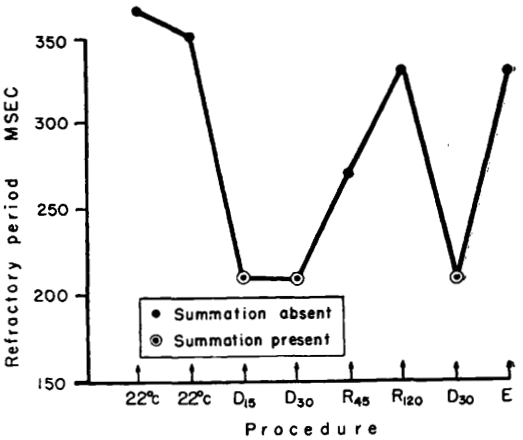

FIG. 4. Relationship between refractory period, summation and action of digitoxin and epinephrine. Temp. 22°C.

D 15	15 min.	after digitoxin
D 30	30 "	" "
R 45	45 "	" wash out with Ringers
R 120	120 "	" " " " "
D 30	30 "	" redigitalization
E	1 "	" epinephrine

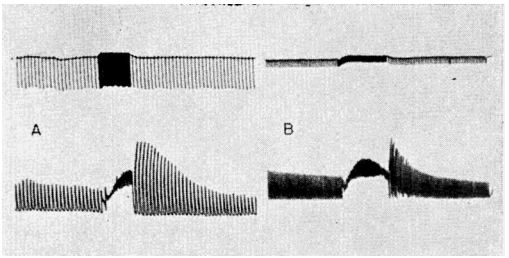

FIG. 5. Summation and post-tetanic potentiation in rat ventricle strip. Stimulus record above, myogram below. Response to stimuli @ 40/sec.
A—After basic rate of 1/sec.
B— " " " " 5/sec.

refractory phase.

While frog ventricle strips could be shown to demonstrate summated responses only under the conditions stated above, rat ventricular strips demonstrated the phenomenon at normal temperature (38°C) and in the absence of treatment with drugs. A typical response is pictured in Fig. 5. Increase in the rate of stimulation from one beat per second to 40 beats per second resulted in summation and incomplete tetanus in this case and in marked post-tetanic potentiation of contractions. Since the normal heart rate of the rat is in the neighborhood of 300 to 400 beats per minute, the experiment was repeated with a driving stimulus of 5 per second. Again, increase in rate of stimulation to 40 per second produced the summated response and post-tetanic potentiation. In contrast with the frog, frequencies of stimulation necessary to produce summation are higher in the rat, 20-40 stimuli per second being optimal, and the response is somewhat less consistent. The results, however, confirm the reports of Robb(5) and of Di Palma and Mascatello(4) on the occurrence of summation in mammalian cardiac muscle.

In view of the observations on the frog it is tempting to consider change in duration of the refractory period as the primary factor in the occurrence of summation and tetanus in rat cardiac muscle. Preliminary experiments indicate that this may not be wholly the case. Of 12 preparations tested for the summated response, 8 consistently gave positive results. No correlation existed between the duration of refractory period and the presence or absence of summation. Moreover, while administration of epinephrine abolished the summated response in all but 3 of these strips, this effect was accompanied by only small and inconsistent changes in refractory period. While these observations are as yet incomplete, they suggest that the operation of other mechanisms, such as the recruitment of contractile elements suggested by Robb(5) may play an essential role in the summation of contractions in rat cardiac muscle with its normally short refractory period.

Summary. 1. Summation of contractions and incomplete tetanus may be consistently produced in frog ventricle strips by elevation of bath temperature. In these experiments a temperature of about 30°C regularly induced the summated response. 2. Digitoxin in conc. of 0.3 μg/ml potentiated the summated response and caused its appearance at lower temperatures. 3. Epinephrine in conc. of 10^{-6} prevented summated response to digitoxin or high temperature. 4. The summated response to digitoxin or high temp. is associated with reduction in duration of refractory period and the inhibitory effect of cooling or epinephrine with increase in duration of refractoriness. 5. Rat ventricle strips may show summation, incomplete tetanus and post-tetanic potentiation at temp. of 38°C and in the absence of treatment with drugs. The mechanisms of these phenomena are not yet delineated.

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