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A STUDY OF INTRACELLULAR POTENTIALS AND CONTRACTIONS IN ATRIA, INCLUDING EVIDENCE FOR AN AFTER-POTENTIAL

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In a previous investigation (Vaughan Williams, 1959*c*) a close correlation was observed between the duration of intracellularly recorded action potentials and the magnitude of contractions of isolated rabbit atria exposed to acetylcholine (ACh), in agreement with the results of Burgen & Terroux (1953). On the other hand, evidence was presented against Burgen & Terroux's suggestion that weakening of atrial contractions in the presence of parasympathomimetic drugs might be due to the failure of a large proportion of contractile elements to respond unless they were activated by an action potential of long duration. Another explanation has to be found for the effect of acetylcholine on contractions, and the hypothesis has been advanced (Vaughan Williams, 1957) that the magnitude of the contractions in atrial muscle is determined by the amount of a substance concerned in activation which is released after the beginning of the action potential, and that this substance is made available for release at a slow rate in the absence of action potentials, and at a faster rate in the presence of action potentials in proportion to their frequency. The hypothesis predicted that when the interval between contractions was long, the size of the contractions would be larger the longer the interval. It further predicted that even at higher frequencies, at which individual contractions became larger ultimately when their frequency was increased (staircase phenomenon), the *initial* effect of the change to a faster frequency would still be to reduce the contractions, and that conversely on returning to the slower frequency, the first contractions would be larger and would then decline towards their original magnitude. The experiments performed to test the hypothesis show that these predictions were fulfilled.

METHODS

The apparatus has already been described (Vaughan Williams, 1955, 1958, 1959*a*). Isolated rabbit atria were mounted horizontally in a bath, through which oxygenated nutrient fluid flowed past them at a constant rate. Contractions were recorded with an RCA 5734 transducer, whose

output was photographed on an oscilloscope or inscribed on a smoked drum with a Kelvin and Hughes magnetic pen. A new method of mounting micro-electrodes (Vaughan Williams, 1959*b*) enabled intracellular potential records to be obtained whose stability over long periods allowed confidence that the potentials were not artificially distorted by the contractions. When the atria were driven electrically the stimuli were applied to the tip of the left atrium, so that action potentials recorded from the left atrium appeared on the tracings before those from the right atrium. In several of the experiments it was necessary to make instantaneous changes in the frequency of stimulation (Figs. 5, 6 and 8). To achieve this it was arranged that when a change was desired, a switch was thrown during the interval between stimuli at the original frequency, whereupon the next stimulus at this frequency operated a fast relay (delay <3 msec), which changed the frequency to any desired new value.

RESULTS

When rabbit atria were exposed to acetylcholine the action potentials shortened and the contractions diminished. If, however, the atria were stopped for increasing periods of time by higher concentrations of ACh, the first beat after

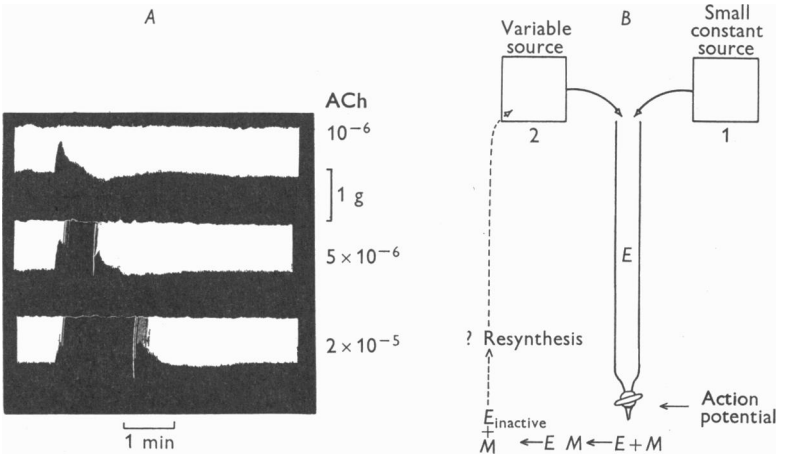

Fig. 1. *A*: Post-arrest potentiation; atrial contractions recorded with transducer and magnetic pen. ACh was introduced as a single amount sufficient to produce the peak concentrations indicated, and was washed out at a steady rate by fluid flowing past the atria. *B*: Diagram of hypothesis described in text, concerning the relation between frequency and size of contractions.

the arrest became larger, and its magnitude was evidently related to the period of arrest (Fig. 1*A*). The action potential which initiated these large beats was still very short (Vaughan Williams, 1959*c*), but was conducted at a fast rate over the whole of the atria. It was as if, during the arrest, some substance involved in the activation of the contractile elements was gradually building up inside the cell. The rate of accumulation was evidently slow, however, because as soon as the atria started beating again, the size of contractions diminished along an approximately exponential course. The observations suggested the hypothesis, formulated diagrammatically in Fig. 1*B*, that the magnitude of the contractions was determined by the amount of a

substance E, which was released during the action potential or in some other way (Evans, Schild & Thesleff, 1958; Axelsson & Thesleff, 1958) so that a reaction occurred during contraction which could be represented as

$$E + M \text{ (muscle contractile element)} = EM,$$

which would then dissociate, into $E_{\text{inactive}} + M$. E_{inactive} might then, of course, be resynthesized to E. It was further suggested that the rate at which E was made available for release was a function of the frequency of the action potentials (variable source, 2, in Fig. 1*B*), but that even in the absence of action potentials it was still made available for release at a slow rate (small constant source, 1). During arrest, when none was being released, the level of E in the reservoir, available for release, would slowly build up, accounting for the phenomenon of post-arrest potentiation when an action potential eventually did arrive, and for the diminution along an exponential course of the first few subsequent contractions.

Relation between frequency and magnitude of contraction

The phenomenon of post-arrest potentiation is illustrated in Fig. 1*A*, in which the contractions were larger the longer the interval of inactivity. This was the reverse of the well-known 'staircase phenomenon', a term which describes an increase in the size of contractions when their frequency accelerates. Analysis of the relative magnitudes of contractions of rabbit atria over a wide range of frequencies has suggested that the relation between contraction and frequency can be divided into four phases, exhibited diagrammatically in Fig. 2. The ordinate represents the size of contractions, the abscissa the frequency of stimulation in beats per minute on a logarithmic scale. At intervals of arrest corresponding to frequencies below about 5–10/min the contractions varied as the interval, illustrating the phase of post-arrest potentiation. In the second phase, at frequencies from about 10–130/min, the staircase effect was evident, contractions rising with frequency. At frequencies above 130/min the contractions changed little (phase 3, 'plateau') until over 220/min, where they began to decline (phase 4, 'overload'). The diagram was constructed from many different experiments, and can only be regarded as a rough guide to the relation between frequency and contraction, because the range of the staircase effect varied considerably in different preparations. The evidence for phases 3 and 4 and part of phase 2 was obtained from atria whose natural pace-maker rate was below the rate of stimulation. Phase 1 was studied in atria whose natural pace-maker had been slowed or stopped by ACh. This procedure was validated on two grounds: (1) At frequencies above the pace-maker rate the same *relative* effect of frequency on contraction was observed whether ACh was present or not (see Fig. 4), although the absolute size of all contractions was smaller in the presence of ACh. (2) The results

agreed with the findings of Vane (1957), who studied the relation of contraction to frequency in isolated left atria which had been severed from their natural pace-maker, and which could therefore be driven at very low frequencies.

The existence of the 'staircase' provided a simple opportunity of testing the hypothesis of Fig. 1*B*. Over the range of frequencies corresponding to phase 2 in Fig. 2, when the frequency was increased the contractions became larger. According to the hypothesis, however, a *sudden* increase in frequency would cause the level of *E* to fall, and contractions ought at first to become smaller, until the rate of supply of *E* was increased in response to the more numerous action potentials. The hypothesis predicts that the initial effect of an increase in frequency would be to reduce contractions, even in the range where a maintained increase in frequency caused a rise in the contractions.

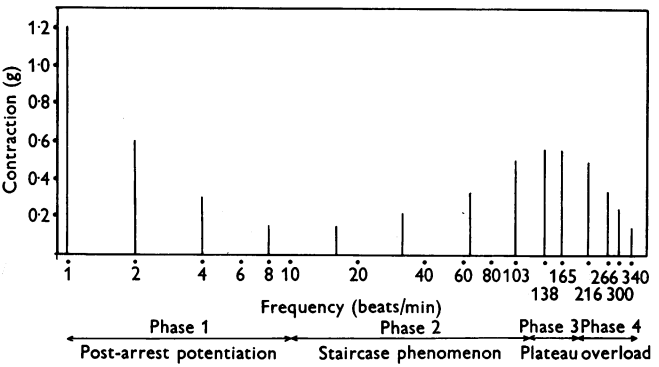

Fig. 2. Composite diagram to illustrate four phases in the relation between frequency and contraction. Ordinate, contraction. Abscissa, frequency of stimulation (log. scale).

An experimental test of this situation is presented in Fig. 3*A*. The upper trace, reading from right to left, gives a record of contractions. Below each contraction is a vertical trace on which is shown a stimulus artifact (S_1) and externally recorded action potentials from the left atrium (LA) and the right atrium (RA). After four beats a second stimulus (S_2) was introduced midway between the S_1 stimuli, so that the frequency of stimulation was doubled. Although after a further ten beats each contraction was now slightly larger than the control contractions, so that the 'staircase' effect was established, the *first* few contractions after the change of frequency were smaller, in accordance with the prediction of the hypothesis. Since each contraction was larger at double the frequency, the atria were now performing more than twice the control amount of work (240%), and according to the hypothesis, when the new steady state was established, *E* must have been supplied to the reservoir at more than double the control rate. Consequently, if the frequency were now changed back, the first contraction after the change ought to be larger than the original controls, since the level of *E* available would now be

higher. The illustration shows that this did in fact occur, and that the contractions subsequently declined along an approximately exponential course towards the control value.

Cause of potentiation

The question arose how the increased frequency ultimately caused more than a doubling of the output of energy. For example, the size of subsequent contractions might be determined by the sum of contractions previously occurring in a given interval of time, i.e. the stimulus leading to an increase in the size

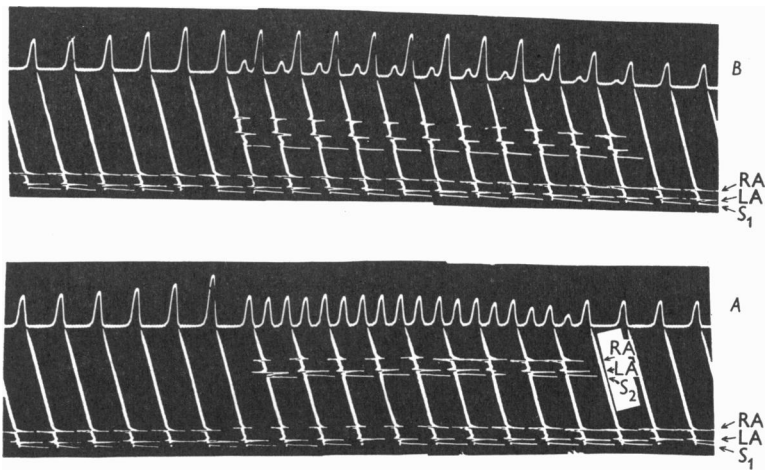

Fig. 3. Effect of doubling frequency on contraction and conduction velocity. Records on moving film, read from right to left. Horizontal record, contractions. Vertical records (read from bottom to top) = records from two pairs of small bipolar external electrodes on left and right atria: S_1, stimulus artifact (from stimulating electrodes on tip of left atrium); LA, action potential from left atrium; RA, from right atrium. The sum of each pair of contractions ultimately reached the same value whether the stimuli were placed at equal intervals (*A*), or unequal intervals (*B*).

of the individual contractions might be something associated with the contractile process itself, such as a product of one of the reactions concerned in contraction. Alternatively, the stimulus leading to the increased output of energy might be associated with the frequency of the action potentials. The experiment shown in Fig. 3*B* represents an attempt to discriminate between these alternatives. In this experiment the frequency was again doubled, but instead of the second stimulus S_2 being placed midway between the S_1 stimuli it was placed just outside the absolute refractory period of the response to S_1. The sum of the last contraction at the slow frequency and of the contraction in response to the first of the intercalated stimuli was larger in *A* than in *B*. Yet the sum of the second pair, and of subsequent pairs, of contractions was almost

identical in the two series, the potentiation ultimately amounting to 242% of the control contraction in *B* as compared with 240% in *A*. This suggested that the stimulus to increased energy output was associated with the frequency of the action potentials rather than with a product of contraction. It may be noted also that conduction velocity in response to the second of each pair of stimuli S_2, in *B*, was much slower than in response to S_1. The fact that the

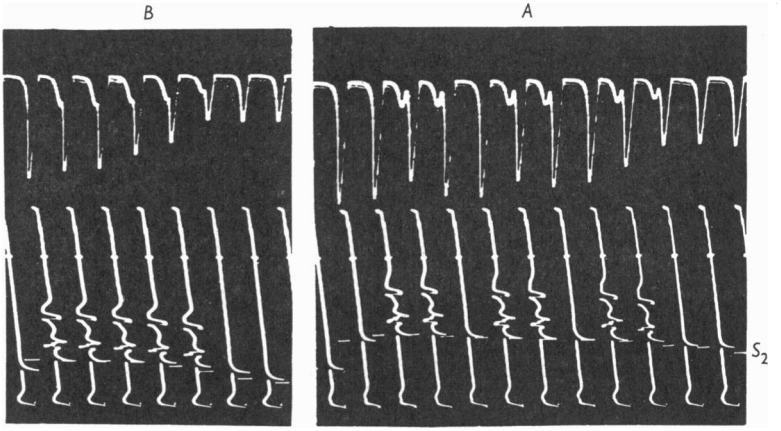

Fig. 4. Experiment to show that acetylcholine did not interfere with the potentiation of contractions by increased frequency. Records as in Fig. 3 except that the responses to S_1 were off the screen, and the responses to S_2 only were seen. *A*: S_2 at first within the refractory period after S_1, then introduced progressively later after S_1 until outside refractory period. *B*: Same procedure as in *A*; between *A* and *B* the atria were exposed to ACh 5×10^{-7}. Note shorter refractory period.

contractile response to the second of each pair of stimuli in *B* was smaller than that to the first was not due to any failure of conduction. Action potentials could be recorded from the whole of the atria. The smaller amplitude of the action potentials in response to the second of each pair of stimuli in *B* is adequately accounted for by greater dispersion as a result of the slower conduction velocity, and to the fact that the overshoot is reduced when a second action potential occurs soon after the refractory period (see Fig. 5*F* and *G*).

Acetylcholine, although it reduced the size of contractions, did not interfere with the phenomenon of potentiation illustrated above. In Fig. 4, also reading from right to left, the upper trace gives contractions, and the vertical traces give the corresponding externally recorded action potentials. At the beginning of the figure on the right (*A*) the atria were being driven at a regular rate, but the action potentials in response to S_1 were off the screen below, in order that a faster sweep speed could be used. A second stimulus (S_2) was then intercalated between the driving stimuli, but at first was placed so soon after the driving stimulus that it was within the absolute refractory period and no

response resulted. The interval between the driving stimulus and the second stimulus was then gradually prolonged, and at the third beat the interval was longer than the refractory period and a response followed. The phenomenon of potentiation was then exhibited as in Fig. 3*B*, the sum of each pair of contractions increasing to 220% of a single control contraction after four double beats, with two single beats in the series. Between Fig. 4*A* and 4*B* the atria were exposed to a concentration of ACh 5×10^{-7}, which reduced the contractions to 68% of the controls. The same procedure as in Fig. 4*A* was then

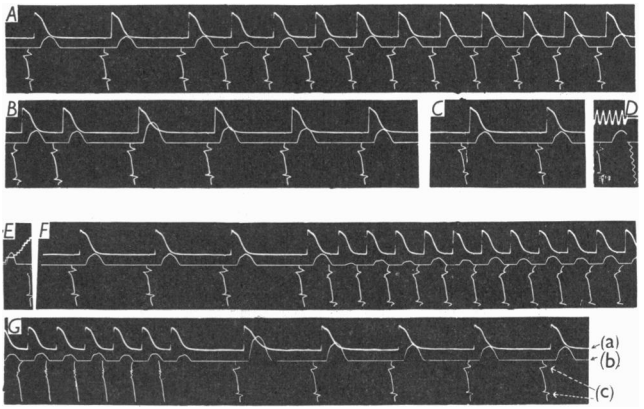

Fig. 5. Simultaneous record of intracellular potentials (upper horizontal trace), contractions (lower horizontal trace), and externally recorded action potentials from left and right atria. Read horizontally from left to right, vertically from top to bottom. *A*, frequency raised from 103 to 188/min. *B*, several seconds later; frequency changed back to 103/min. *C*, 1 min later. *D*, 20 c/s horizontally; 100 c/s vertically. *E*, steps of 10 mV. *F*, records from another fibre in same preparation; frequency raised from 103 to 266/min. *G*, return to 103/min. (*a*) Intracellular action potentials. (*b*) Contractions. (*c*) Externally recorded action potentials from left and right atria.

repeated; the refractory period was now much briefer, because ACh shortens the action potential, but the phenomenon of potentiation was still evident, the sum of contractions increasing to 280% after four double beats. Thus it was concluded that if action potentials were able to stimulate the output of energy available for contraction they could do so equally well whether they themselves were of short or long duration.

Intracellular potentials

The changes in size of contraction and in conduction velocity produced by variations in frequency made it of interest to determine what simultaneous changes might be occurring in the shape of the intracellular action potentials. In Fig. 5, reading from left to right, the upper horizontal trace records intracellular action potentials. The horizontal trace below it records contractions,

and the vertical traces the corresponding externally recorded action potentials. In Fig. 5*A* the atria were being driven at 103 beats/min and the frequency was suddenly increased to 188/min. Each contraction ultimately increased by 12%, so that Fig. 5*A* illustrates the operation of the staircase phenomenon, and the work done increased to 224%.

The resting potential fell from 73·5 to 71 mV when the steady state at the new frequency was reached, and the overshoot from 29·5 to 26·5 mV. Both recovered to their original values when the frequency was changed back to the control rate of 103/min. Conduction velocity decreased when the frequency was raised. In contrast to the contractions, which were at first smaller, but then rose to a new and higher level, the decrease in conduction velocity was apparent from the very first beat at the higher frequency, and continued to decrease until the new steady state was reached. Similarly, on returning to the control frequency conduction velocity immediately increased again on the first beat at the lower frequency. In other runs on the same preparation it was found that the rate for maximum size of contraction was reached at 145/min, and thereafter the size of contractions altered little until the rate reached 210/min. It is of interest that in intact rabbits e.c.g. records showed a natural rate of 200–220/min. In so far as comparisons with conditions *in vivo* are permissible, after allowing for the difference in temperature (the rate increases approximately 10 beats/min per degree centrigrade), and better oxygenation, it would seem probable that the natural rate would be in the 'plateau' region of Fig. 2, and the 'staircase' phenomenon is not likely to have much significance in life.

In Fig. 5*F* the rate was increased from 103 to 266/min. At this frequency the phase of overload had been reached, and the individual contractions were reduced by a third. The work done increased to only 187%, in comparison with the increase to 224% when the rate was raised to 188/min. Conduction velocity became so slow that the action potential from the right atrium eventually went off the screen, but returned on the first beat when the control frequency was restored. The resting potential fell from 73·5 to 69 mV, and the overshoot from 30 to 22 mV.

Detailed comparisons were made in several runs of the effect of frequency changes on contraction and on the duration of the action potential, measured as follows: (*a*), half-time for repolarization; from the peak height of the intracellular action potential to half-way towards the full resting potential; (*b*), duration of repolarization; from the peak height to within 4 mV of the full resting potential (this point was chosen arbitrarily, because it was difficult to measure the exact moment of return to the full resting potential). To illustrate the stability of the phenomena observed, measurements of the duration of repolarization (*b*) and of contraction have been plotted in Fig. 6 from the experiment of which extracts have been presented in Fig. 5*A–C*, and from

another run in which exactly the same frequency changes were made. The ordinates represent the duration of repolarization in milliseconds and the size of contractions in milligrams, and the abscissae elapsed time in seconds. It is evident that, when the frequency was returned from the fast to the slow rate, both the contractions were larger and the duration of the action potential was longer, but that the percentage change in duration was less than the percentage change in the contraction. This suggested that the contraction itself was associated with an after-potential added to an action potential of fixed length.

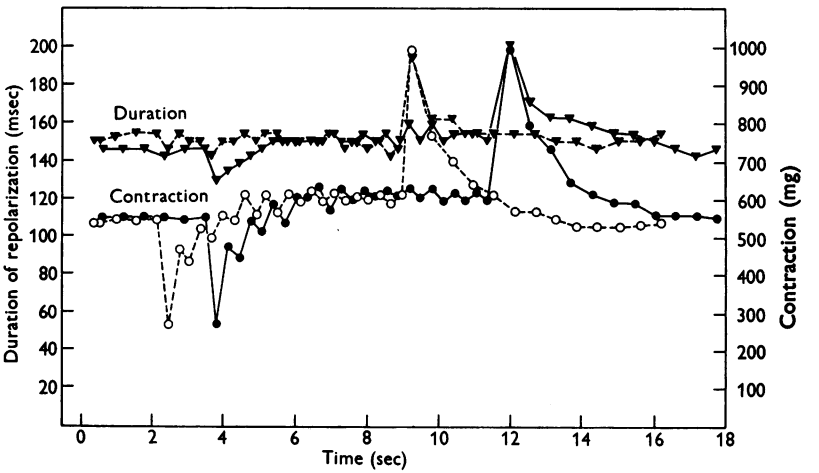

Fig. 6. Experiment to illustrate stability of relation between frequency and contraction. Ordinates, duration of repolarization, and size of contraction; abscissa, time. —— effect of a change of frequency from 103 to 188/min and back again: ---- effect of an exactly similar change of frequency 2 min later. The micro-electrode was in the same fibre throughout.

In Fig. 7 photographs of contraction and intracellular potential during the last beat at the faster frequency (dotted lines) have been superimposed on photographs taken during the first beat after restoration of the control frequency. It is clear that only the tail of the action potential was prolonged.

In Fig. 8 contractions and various parameters of the intracellular action potentials have been plotted, to demonstrate the effects of 'overload' when the frequency was raised from 103 to 266/min, in a run repeating the conditions of the experiment illustrated in Fig. 5*F–G*. In Fig. 8*A* changes in contractions have been compared with changes in the duration of repolarization and in the half-time for repolarization. The percentage changes in half-time were even smaller than the changes in the duration of repolarization, confirming the view that the early part of the action potential was not changed. In Fig. 8*B* the associated changes in resting potential and total height of the action potential (the difference between them giving the overshoot) have been plotted. Two points are evident. First, that the stability of the measurements

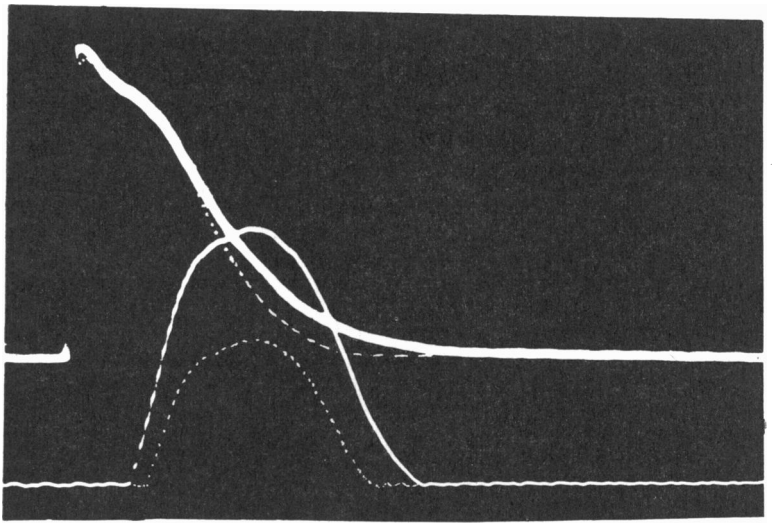

Fig. 7. Records indicating that the longer duration of repolarization associated with a larger contraction could be due to an after-potential. Superimposed records of contraction and intracellular potential during the last beat at the fast frequency (dotted line) and the first beat on returning to the control frequency, taken from one of the experiments shown in Fig. 6.

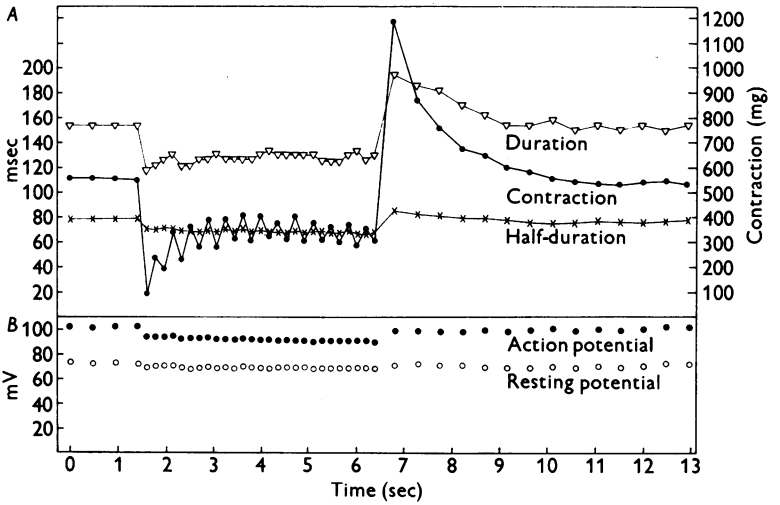

Fig. 8. Effect of a high frequency on contraction and intracellular potential. *A*: The percentage changes in the duration of repolarization were less, and in the half-time of repolarization even less still, than the changes in contraction in response to an alteration in frequency from 103 to 266/min and back. *B*: Associated changes in the resting potential, ○, and the total height of the action potential, ●. There was a relatively big reduction of the overshoot potential.

was such that although the changes were small, they were highly significant. Secondly, that the changes in the overshoot occurred so rapidly, on the first beat at each new frequency, that it seemed unlikely that the fall in the overshoot could have been due to an accumulation of sodium inside the fibre.

In order to confirm the supposition that the contraction was associated with the addition of an after-potential to a finite action potential, in Fig. 9 the contractions were plotted against the duration of repolarization and half-time for repolarization at various frequencies in two experiments. The regressions were

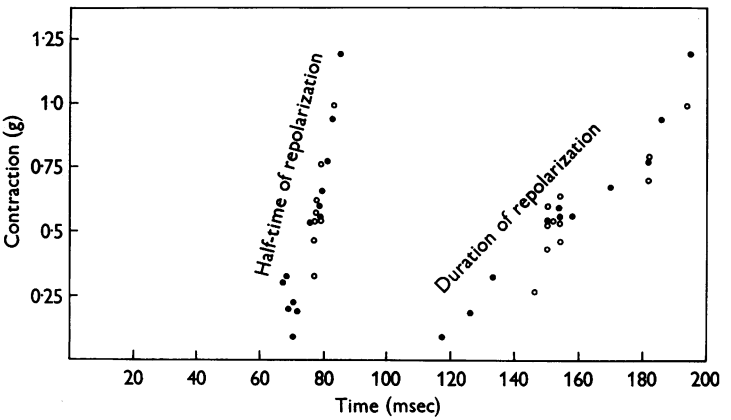

Fig. 9. The duration of repolarization and the half-time of repolarization plotted against the size of contraction in two experiments.

reasonably linear, and when the contraction was extrapolated to zero, it was evident that the after-potential was superimposed upon a 'basic' action potential whose half-time for repolarization was about 70 msec and whose duration was about 110 msec. This observation, that the changes in duration of the action potential associated with contraction were confined to its terminal part, ruled out the possibility that the simultaneous diminution of contractions could be responsible for the effect on the duration of the action potential produced by ACh, which characteristically altered the shape of its early part. Even when contractions are still occurring the half-time for repolarization in the presence of ACh may be only 5–8 msec. This point is well illustrated by Fig. 2*C* from a previous paper (Vaughan Williams, 1959*c*) which shows an intracellular potential recorded during the first beat after a period of arrest by ACh. Here the half-time for repolarization was less than 10 msec, but the action potential ended in a 'tail' of low voltage, which is in accord with the present evidence, since a large post-arrest contraction would be expected to be associated with an after-potential.

DISCUSSION

Simultaneous measurements of contraction, intracellular potential and conduction velocity were made in isolated rabbit atria both beating spontaneously and stimulated at different frequencies. When the atria were stopped by acetylcholine (ACh) for increasing lengths of time, it was found that the first contraction after the arrest became larger the longer the interval. The hypothesis suggested itself that during the period of arrest some substance involved in activation was building up inside the fibre at a slow but fairly constant rate, and that the size of the contraction was determined by the amount of this substance available for release by an action potential or other initial event. Extending the hypothesis to normal contractions it was further suggested that the amount of the substance made available might be augmented when the frequency of stimulation was increased.

An attempt has been made to test this hypothesis by changing the magnitude of individual contractions by stimulation at widely different frequencies. At frequencies below 5–10/min (phase 1) contractions were larger the longer the interval between stimuli, in agreement with the hypothesis. At frequencies between about 10 and 130/min (phase 2), although contractions ultimately became larger when the frequency increased (staircase phenomenon), the *initial* effect of an increase in frequency was to reduce the size of contractions, again in agreement with the hypothesis. Above 130/min (phase 3), there was little change in the size of contractions until about 230/min, whereafter the contraction began to decline (phase 4). Trautwein & Dudel (1954) studied the effects of frequency on contraction in isolated papillary muscle from cat ventricle, and found that contractions increased with increasing frequency up to about 280/min, after which they declined. Phase 2 thus passed directly into phase 4. They did not observe phase 1, perhaps because they only employed frequencies above 30/min. Hollander & Webb (1955), using rat atria, found that contractions declined when the frequency of stimulation was raised from 200 to 420/min, but they did not investigate the effect of frequencies below 200.

The question arose whether the stimulus to an increased energy output came from something associated with the contractile process, i.e. was related to the sum of previous contractions in a given interval of time, or from the increased number of action potentials. When the frequency was doubled by giving paired stimuli, the potentiation of contraction was ultimately the same whether the stimuli were spaced at equal or unequal intervals, even though the sum of the contractile responses to the first pair of equally spaced stimuli was greater than to the first unequally spaced pair, implying that the factor of importance was the frequency of the action potentials. The conclusion drawn was that in response to an action potential a finite amount of energy was made available

for contraction, but if it was not used in contraction, it was still available to be added to the amount released by the succeeding action potential. This hypothesis provided a simple explanation for the results of Hoffman, Bindler & Suckling (1956), who found that the potentiation of a cardiac contraction by a preceding extrasystole was greater the earlier the extrasystole occurred after the previous normal contraction; i.e. the smaller the mechanical response to the extrasystole, the larger the potentiation of the next contraction.

Intracellular records

The duration of the intracellularly recorded action potential was correlated with the size of contraction. The shape of the early part of the action potential was unchanged, the lengthening being confined to the addition of an after-potential to a 'basic' potential with a half-time of about 70 msec and a duration of 110 msec. The stability and reproducibility of the after-potential argue against its being a contraction artifact. Hollander & Webb (1955) may also have observed this phenomenon. They stated 'marked variations of potential were often seen near the end of repolarization. One might interpret these as negative after-potentials of less than 5 mV, but it is more likely that they were artifacts produced by local disturbances.' They nevertheless concluded that there *was* a correlation between the size of contractions and the duration of the action potential, but decided that the longer potentials were the cause of the larger contractions and not vice versa. In this respect, there would seem to be a clear difference between atrial and ventricular muscle. Hoffman & Suckling (1954) found that in the papillary muscle of the dog heart the duration of the intracellular action potential did not vary at frequencies below 60/min, but that at frequencies above this the action potential became steadily shorter. They did not measure contractions simultaneously, but Trautwein & Dudel (1954) did so in cat papillary muscle, and showed a decrease in the duration of the action potential between 30 and 400 beats/min, so that the action potential was becoming shorter as the contractions became larger. It is possible that any after-potential associated with contraction in ventricular muscle is masked by the large 'plateau' of the ventricular action potential.

In contrast to the complex relation between frequency and contraction, conduction velocity was always slower when the frequency increased. It was observed also that the rate of rise of the action potential was slower. At high rates, in phase 4, the overshoot potential was reduced and the resting potential fell by a few millivolts. It was improbable that the fall in the overshoot was due to an accumulation of intracellular sodium, because the overshoot was reduced on the first beat after a change to a fast frequency and was restored on the first beat after changing back to the control frequency.

The hypothesis discussed above would offer a point of departure for an explanation of vagal and sympathetic activity. Noradrenaline, by taking the

brake off carbohydrate break-down in accordance with biochemical evidence, might proportionately increase the energy made available for contraction in response to each action potential. Conversely ACh, by reducing the proportion of activator released, could diminish the absolute size of all contractions without interfering with their relative potentiation when action potentials were more frequent (Fig. 4). This would accord with the views of Sarnoff (1955) that small changes in sympathetic and parasympathetic tone have much more important effects on the work output of the heart than changes in the diastolic length of the fibres.

SUMMARY

1. Measurements were made of the effects of changes in the frequency of stimulation on contraction, intracellular potential and conduction velocity in isolated rabbit atria.

2. The relation between frequency and contraction was divided into four phases. Below 10/min contractions were larger the slower the frequency (post-arrest potentiation). Between 10 and about 130/min contractions were larger the faster the frequency (staircase phenomenon). At higher frequencies a plateau was reached when there was little change in contraction, until the rate reached about 220/min, whereafter the contractions declined.

3. At high frequencies there was a small but significant fall in resting potential, and a much larger fall in the overshoot potential.

4. The relation of stimulation frequency to conduction velocity was simple. Over the whole range, the faster the frequency the slower the conduction velocity.

5. The duration of the repolarization was correlated with the size of contraction. Evidence was obtained that this was due to an after-potential being added, in proportion to the magnitude of contraction, to a 'basic' action potential with a half-time of approximately 70 msec and a duration of 110 msec.

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