J. Physiol. (1959) 147, 325–332

SIMULTANEOUS MEASUREMENTS OF CONTRACTIONS AND INTRACELLULAR POTENTIALS IN ISOLATED RABBIT ATRIA EXPOSED TO ACETYLCHOLINE

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(*Received* 20 *February* 1959)

There have been several hypotheses concerning the manner in which vagal stimulation or parasympathomimetic substances may weaken the contractions of cardiac muscle, or produce a negative inotropic effect, as some authors prefer to say. Muskens (1898) examined the effect of vagal stimulation on the frog heart, and concluded that 'the diminishing force of contraction under vagal inhibition may mean that fewer and fewer fibres take part in the contraction, because the decreasing power of conduction prevents more and more fibres from being reached by the excitation waves'. Mines (1914) studied the effects of vagal stimulation on the frog heart, eliminating changes in rate by applying atropine locally to the sinus venosus, and observed that contractions diminished in the absence of any failure in conduction. In his words, 'does the weakened contraction under vagus inhibition mean the contraction of fewer fibres, through failure of the excited state to reach the rest, or does it mean the weakened contraction of all or most of the fibres?' Mines favoured the latter view. He observed also that the diminished contractions were associated with a shortened duration of the action potential.

In mammalian atria there is no evidence of 'decremental conduction' after vagal stimulation or exposure to acetylcholine (ACh). On the contrary, conduction velocity in isolated rabbit atria is increased at a time when contractions are reduced (Vaughan Williams, 1954, 1958). Burgen & Terroux (1953) revived in a modified form the view that the contraction of fewer elements might be responsible for the diminution of contractions in the presence of parasympathomimetic drugs. They made simultaneous measurements of contractions and intracellularly recorded potentials and found that the action potentials were shortened, and that the reduced contractions had earlier peaks. They stated that 'during the progressive inhibition of contraction by carbamylcholine (CCh) there is a definite correlation between the shift in time of peak tension and the height of the tension curve, which suggests that

the duration of action potential necessary for excitation may differ for different *myofibrils*, so that with increasing brevity of the action potential the more slowly responding fibrils fail to contract'. 'The effect of ACh or CCh would thus be to eliminate progressively that part of the contraction which is due to the later responding elements.' In the present paper some evidence is reported which is relevant to the above hypotheses.

METHODS

The apparatus has already been described (Vaughan Williams, 1955, 1958, 1959). Rabbit atria were placed horizontally in a bath, the temperature of which was controlled at 31° C to within ±0·1°. Contractions were recorded with an RCA 5734 transducer, and displayed on one beam of a Dumont 322 oscilloscope. Intracellular potentials were displayed on the other beam. External potentials, from which conduction velocity was calculated, were recorded from right and left atria with pairs of small platinum bipolar electrodes, and were displayed on a Cossor 1049 oscilloscope. When the atria were beating spontaneously, the action potential from the right atrium triggered the sweep. The screens of both oscilloscopes were photographed simultaneously with a Grass camera; the time at which each exposure was made was also recorded to within 0·1 sec. When the atria were driven electrically, Ag–AgCl electrodes were placed on the tip of the left atrium. All intracellular records were taken from the endocardial surface of the right atrium, some distance from the natural pace-maker. The Dumont oscilloscope sweep was itself made to produce a pulse at the end of its traverse which set in motion the mechanism for shifting one frame in the camera; this in turn operated a shutter to record the time. Consecutive records could thus be obtained on stationary film, the frames being changed automatically between each heart beat.

RESULTS

It was already known (Mines (1914) in the frog; Burgen & Terroux (1953) in the cat) that the diminution of the contractions of atria during vagal inhibition or during exposure to parasympathomimetic substances was associated with a shortened duration of the action potential. In considering the possibility of a causal link between the two phenomena it was of interest to determine how close was their association. Simultaneous observations of contractions and intracellular potential have been made during thirty-two exposures to various concentrations of ACh in eight pairs of rabbit atria. For comparisons between potential and contraction to be significant, however, it was necessary that the micro-electrode should remain in a single muscle fibre during a control period and thereafter during the inhibition of contractions by ACh and during the recovery period. Only five experiments could be regarded as satisfactory by the criteria discussed in a previous paper (Vaughan Williams, 1959) and the results of these have been plotted in Fig. 1. The ordinate shows the peak size of contractions in milligrams, and the abscissa the time in milliseconds taken for the intracellular action potential to recover from its peak to half-way towards the full diastolic resting potential. The correlation is sufficiently good for a causal connexion between the shortening of the action potential and the diminution of contractions to remain a possibility, or at least not to be ruled out as an impossibility.

Burgen & Terroux (1953) had observed not only that the action potential was shorter when the contractions were smaller in the presence of CCh, but also that the time from the beginning to the peak of the reduced contractions was shorter than in normal contractions. This led them to the hypothesis that some myofibrils required a long action potential in order to be excited, and consequently failed to contract when the action potential was short. It was, therefore, of interest to learn what the duration of the action potential would be in a situation in which contractions were larger than normal, and in which it might be presumed that all or most of the myofibrils were active.

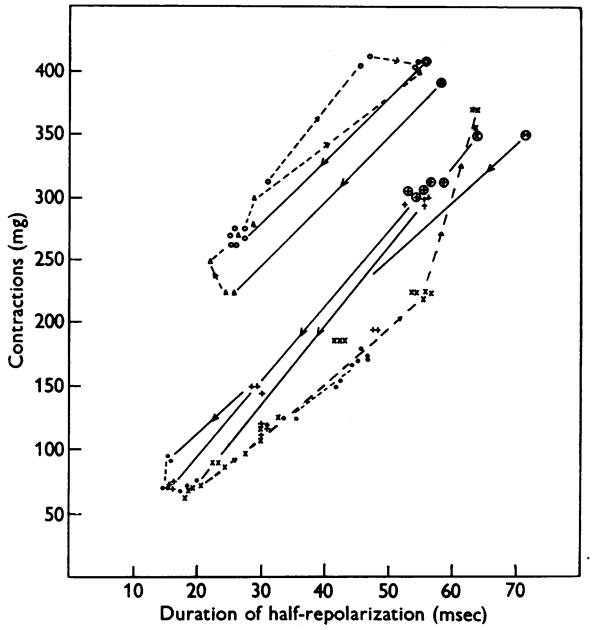

Fig. 1. Correlation between contractions and half-time for repolarization. Different symbols indicate the results of five experiments from four pairs of atria; control observations have been ringed, and the order in which subsequent observations were made has been indicated by arrows.

It is well known that if the atria are arrested by vagal stimulation or by ACh, the first contraction after the pause is larger than normal. This is the reverse of the 'staircase' phenomenon and is illustrated by a smoked-drum record of contractions in Fig. 2*A*. During the phase of inhibition the action potential became very short, the half-time for repolarization being reduced from about 60 msec to as little as 7–10 msec. It was of interest to know whether during the large first contraction the action potential was still short, or had lengthened in proportion to the increase in the contraction. Many attempts were made to record intracellular action potentials and contractions simultaneously in these conditions, but unfortunately the electrode was always

pulled out of the fibre by the very large contraction. It was possible, by physically restricting the movement of the atria and so sacrificing the contraction record, to show that the action potential during the first beat after arrest by ACh was short. Fig. 2*B* shows control action potentials before the addition of ACh to the bath. Between *B* and *C* the auricle was stopped for 70 sec by the addition of ACh 5×10^{-5}, and *C* shows the first action potential

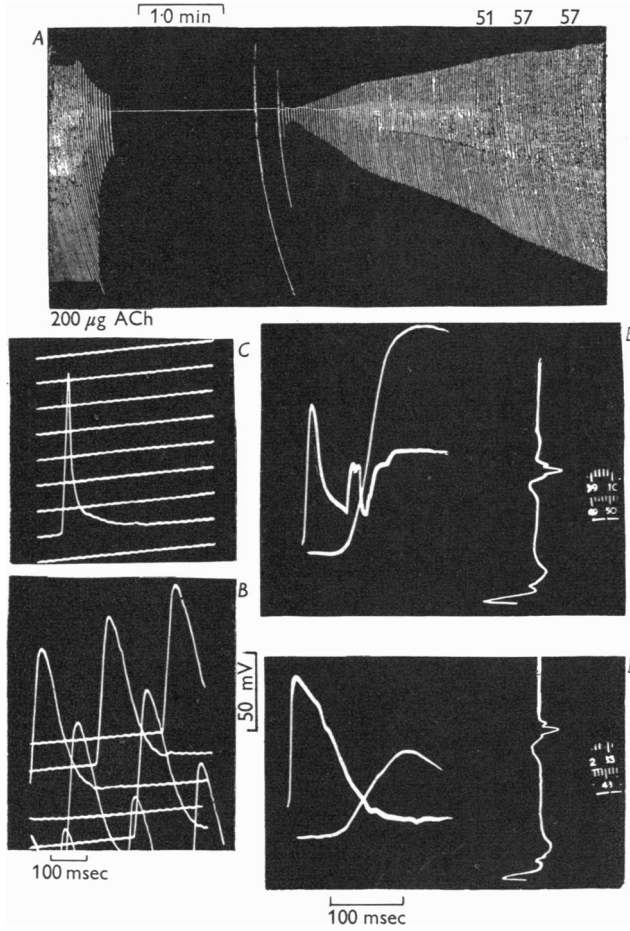

Fig. 2. Arrest of contractions by ACh and recovery. *A*: Smoked drum record to illustrate the large first beat after arrest. *B* and *C*: Records of intracellular potentials taken on moving film before exposure to ACh and after arrest; the first action potential after arrest was short, but there was no contraction record. *D*: Controls; upper trace, intracellular potential; lower trace, contraction; vertical trace on right, action potentials recorded from right and left atria with bipolar surface electrodes, from which conduction velocity could be calculated; inset, time. *E*: First contraction after arrest by ACh for 96 sec. The micro-electrode was pulled out of the fibre by the large contraction, but it was evident that the action potential was short. Note increased conduction velocity.

after the arrest, but in the absence of a contraction record there was only presumptive evidence that the associated contraction would have been larger than normal. Partial success in obtaining simultaneous mechanical and electrical records was eventually achieved on two occasions, illustrated in Fig. 2*E* and in Fig. 3*D*. Although there was evidence that the electrode was pulled out of the fibre by the abnormally large contractions and there was some fall of the resting potential, the electrode remained in the fibre long enough to indicate that

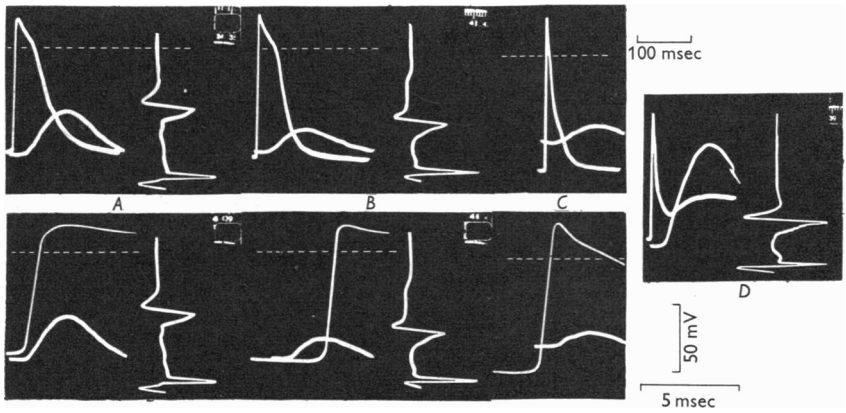

Fig. 3. Records of contractions and intracellular and extracellular potentials during inhibition by ACh and after arrest. *A*: Controls; the upper trace in each frame records the intracellular potential, the lower trace the contraction. The vertical trace records action potentials from bipolar surface electrodes on right and left atria. The dotted lines indicate the zero potential before the micro-electrode was inserted into the fibre. In the lower frames the sweep speed of the trace recording the intracellular potential was much faster, for more accurate measurement of the rate of rise of the action potential. *B* and *C*: Records taken from the same fibre during the action of ACh 2×10^{-5}; the resting potential, the overshoot potential, the rate of rise of the action potential and conduction velocity had all increased. *D*: Record during the first beat after the atria had been arrested for 70 sec.

the action potential was still short. It may also be noted that the peak of the large contraction did not occur earlier than normal. Thus a large contraction in which all or most of the contractile elements were probably active was produced in response to an action potential of very short duration. This would seem to make it less likely that the diminution of contractions by ACh was due to the inability of a substantial proportion of the myofibrils to respond to a short action potential. It might still be maintained on other grounds, of course, that the inhibition of contractions was due to the failure of many contractile elements to be activated, but not on the grounds of an association between a short action potential and an early peak in the reduced contraction, because it is clear that a short action potential can also be associated with a large contraction without an early peak.

It has been suggested (Vane, 1957) that a large part of the inhibition of

contractions by ACh may sometimes be attributed to the simultaneous slowing of the rate, as a consequence of the 'staircase' effect. In the present and previous experiments, however (Vaughan Willliams, 1958), it was found that contractions diminished to the same extent whether the frequency of the contractions was allowed to diminish or was kept constant by electrical stimulation. If atria were exposed to ACh in such a way that the drug arrived at the pace-maker last, a large diminution of contractions nevertheless occurred before there was much change in frequency (Fig. 4*A*). If, however, the ACh

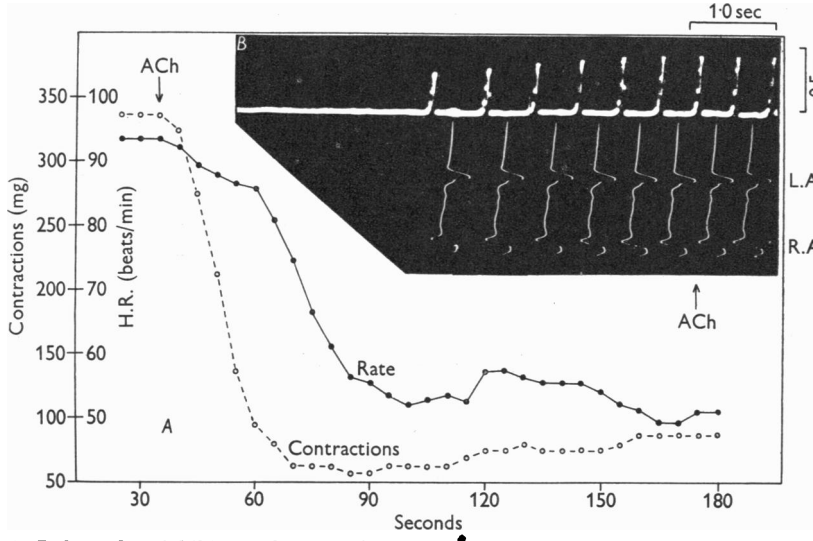

Fig. 4. Independent inhibition of pace-maker and contractions by ACh. *A*: Ordinates, contraction and rate of beat; abscissa, time. At the arrow ACh was introduced to the bath to give a final concentration of 10^{-6}, but the flow was arranged so that it reached the pace-maker last. *B*: Upper horizontal trace, record of contractions on moving film (read from right to left); vertical traces, records of action potentials from right and left atria. At arrow, ACh sufficient to give final concentration of 10^{-5} directed towards pace-maker region; the atria were arrested when contractions were reduced by only 30%.

was directed first on the pace-maker, the atria stopped beating before there had been much diminution in the contractions and when conduction from the right to the left atrium was unaffected (Fig. 4*B*). Webb (1950) stated 'when doses (of ACh) were large, the auricles were stopped when the amplitude fell to zero, the pace-maker in all probability continuing to discharge impulses'. To test this possibility atria were stimulated during the period of arrest by ACh. Even in the presence of concentrations as high as 1 mg/ml., the atria always responded with a contraction. It was concluded, therefore, that the atria were stopped by ACh because of the absence of impulses from the pace-maker, and not as a consequence of a complete inhibition of the contractile mechanism (cf. Hoff, 1955; Hutter & Trautwein, 1956).

DISCUSSION

Simultaneous measurements have been made of contractions and intracellular potentials in isolated rabbit atria. It was found that during inhibition of contractions by acetylcholine there was a correlation between the magnitude of contractions and the duration of the action potential (measured as the half-time for repolarization), in agreement with observations made by Burgen & Terroux (1953) of the effect of carbamylcholine on strips of cat atria. These authors had also observed that the small contractions, associated with short action potentials, reached their peaks earlier than normal contractions. They suggested that a substantial proportion of myofibrils required action potentials of long duration in order to become activated, and consequently failed to be excited when the action potentials were short. It was concluded that the short duration of the action potential in the presence of CCh was causally linked to the negative inotropic action of this substance.

It is well known that the first contraction of cardiac muscle after a period of arrest is larger than normal. In the present experiments it was found that when atria were arrested by high concentrations of ACh the first large contraction occurring after the pause was associated with a very short action potential. This contraction, sometimes more than twice the magnitude of control contractions observed before exposure to ACh, had a peak which was not earlier than that of a normal contraction, and was, therefore, presumably produced by activity in all or most of the myofibrils. Thus, although it might still be maintained on other grounds that the diminution of contractions by ACh was due to the excitation of fewer elements, this view was difficult to maintain on the grounds of the association of short action potentials and small contractions with early peaks, since large contractions without early peaks could also be initiated by short action potentials.

If atria were stimulated electrically during the period of arrest by ACh, they always contracted even in the presence of concentrations as high as 1 mg/ml. Further, if atria were exposed to ACh in such a way that the drug reached the pace-maker last, large diminutions in contractions occurred before there was much change in rate. Conversely, exposure of the pace-maker to ACh caused arrest of the atria before there was much diminution of contractions or any effect on conduction from the right to the left atrium.

It was concluded that the *arrest* of rabbit atria by ACh was due to the absence of potentials from the pace-maker and not to the total inhibition of the contractile process, and that the effects of ACh on rate and on contraction were largely independent. Further it would seem that the undoubted association between the diminished contractions and the shortening of the duration of the action potential may be coincidental. The effects of ACh on contractions and on action potentials may both be secondary consequences of a more fundamental action.

SUMMARY

1. Simultaneous measurements of contractions and intracellular action potentials have been made in isolated rabbit atria in the presence of various concentrations of acetylcholine.

2. It was found that the diminution of contractions by ACh was correlated with the reduction in duration of the action potential.

3. If the atria were arrested by ACh, the first contraction after the pause was very large, without an earlier-than-normal peak, but was associated with a short action potential. This makes less probable, as an explanation of the negative inotropic effect, the suggestion that a large number of myofibrils require a long action potential to activate them and therefore fail to be activated by a short action potential.

4. Evidence was presented of the independence of the diminution of contractions and the slowing of the rate by ACh.

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