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SOME OBSERVATIONS CONCERNING THE MODE OF ACTION OF ACETYLCHOLINE IN ISOLATED RABBIT ATRIA

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Previous studies of the effects of vagal stimulation or of acetylcholine upon conduction velocity in mammalian atrial muscle have not always led to the same conclusions. Lewis, Drury & Bulger (1920) observed in the dog no change in conduction velocity after vagal stimulation, but Brooks, Hoffman, Suckling & Orias (1955) reported that conduction velocity was increased. Burgen & Terroux (1953*b*) observed in the cat a decrease in conduction velocity in strips of auricle exposed to ACh and carbamylcholine, but Ramos & Rosenblueth (1947) found an increase, which they attributed to the shortening of the duration of the action potential. A preliminary investigation in rabbit atria (Vaughan Williams, 1954) had shown that ACh sometimes had a diphasic effect on conduction velocity, the usual increase in velocity occasionally being preceded or interrupted by a temporary phase of reduced velocity. The present paper is largely devoted to an attempt to explain these effects in terms of the changes produced by ACh in the various parameters of the action potential recorded intracellularly.

METHODS

Isolated rabbit atria were suspended horizontally in apparatus which has already been described (Vaughan Williams, 1955; Marshall & Vaughan Williams, 1956); the temperature was 30-31° C, except where otherwise stated. Conduction velocity was measured with bipolar platinum electrodes. Intracellular records were obtained with conventional methods, the input valve being an ME 1400 mounted on the micromanipulator. The input time constant was 40 μ sec, measured by injecting a square wave into the bath earth line, with an 11 M Ω micropipette just touching the surface of the solution (capacity = 4 pF). In order to measure the rate of rise of the upstroke of the intracellular action potential, a high speed of the oscilloscope sweep was necessary, and the following procedure was adopted to obtain photographs on stationary film of the required part of the trace, when the auricles were beating spontaneously. An external electrode was placed on the pace-maker, and the action potential from this was used to trigger the sweep. The micro-electrode was inserted some distance away, the conduction time from external to internal electrode providing the necessary delay. A pulse was then taken from the end of the sweep and used to operate the camera movement. As soon as the film was again stationary another pulse operated a

shutter exposing a clock face, to give the time of exposure accurate to within 0.1 sec. Changes in the rate of the pace-maker could thus be calculated from beat to beat, and the camera simply followed the atrial rhythm, thus avoiding much waste of film when the pace-maker was arrested for long periods.

Solution flowed continuously past the preparation at a rate which ensured complete removal within 15 min of a dye introduced as a test substance. Acetylcholine was added either to the bath in a single amount in a small volume, so that the concentration started high and then fell to zero within a few minutes; or to the supply reservoir so that the auricle was exposed to a more constant concentration. In the earlier experiments contractions were recorded by a lever writing on a smoked drum, but in the later experiments, to obtain absolute accuracy of synchronization, the contractions were measured by an RCA 5734 transducer, whose output was photographed with the corresponding action potentials on the same film.

RESULTS

The effects on conduction velocity of various concentrations of ACh were observed in 110 experiments on twenty-nine isolated rabbit atria. In a few experiments the higher concentrations of ACh induced irregularities of electrical activity which temporarily invalidated measurements of conduction velocity. These irregularities could be classified into three categories.

- (a) A change in the site of the pace-maker. One of the bipolar electrodes was placed on or near the pace-maker, so that a change in the site of origin of electrical activity was shown by a change in the shape of the first recorded action potential. A change of pace-maker was rare, occurring in only two auricles.
- (b) A 'saltatory' change in the pathway of conduction. In nine experiments in five auricles it was observed that the second action potential, without changing its shape, suddenly became much closer to the first (Fig. 1*B*). This change occurred in the interval between one beat and the next, and was so large that it was concluded that it was not due to an increase in conduction velocity, but to the impulse jumping a whole group of fibres instead of being conducted along them. This saltatory effect was never observed with concentrations of ACh lower than 10^{-6} . In most auricles it was never seen, even with concentrations sufficient to stop the pace-maker for many seconds.
- (c) Development of an ectopic pace-maker. In two experiments, although the regular frequency and shape of the first action potential was unaltered, indicating that there was no change in the original pace-maker, the second action potential exhibited an independent frequency, implying that the part of the atrium under the second electrode had broken away from the original pace-maker and was following an ectopic focus. This phenomenon is illustrated in Fig. 1*C*. Here the second action potential, whose normal shape was that shown in Fig. 1*C*, 3, became inverted when it no longer followed the original rate (Fig. 1*C*, 1, 2, 4) implying that the ectopic focus was on the opposite side of the electrode from the true pace-maker.

In practice these changes in pace-maker or pathway, which could have invalidated conclusions about changes in conduction velocity, were usually not difficult to recognize owing to the suddenness of their occurrence and the concomitant changes in shape of the action potential (cf. Burgen & Terroux, 1953*b*). Moreover, true changes in conduction velocity still occurred even when calculated from a new pace-maker or via a different pathway. The usual effect of ACh was to produce a change in conduction velocity which

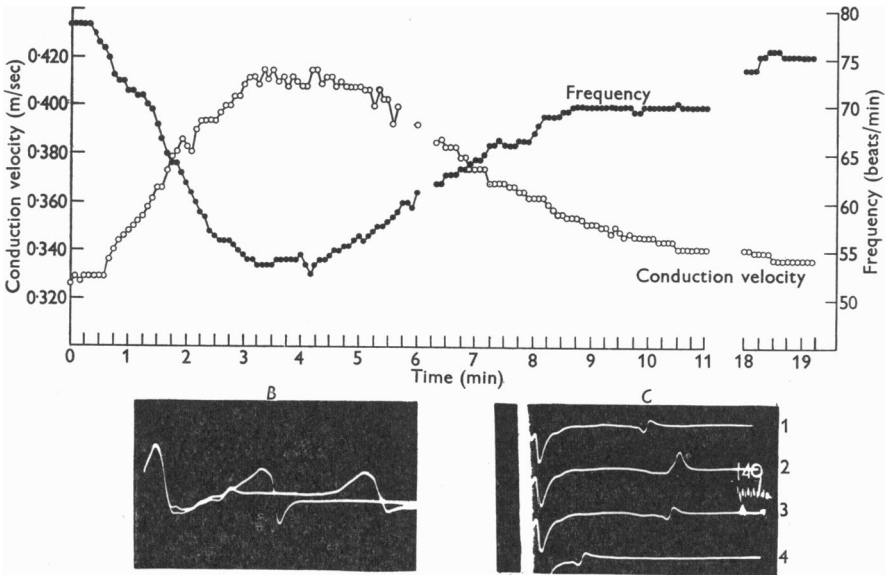


Fig. 1. *A*: simultaneous record of the effect of ACh on conduction velocity (O) and frequency of contractions (●). *B*: saltatory conduction without change of site of pace-maker. *C*: region under second electrode no longer following original pace-maker in 1, 2 and 4.

developed gradually from beat to beat and followed a characteristic time course during the inhibition and recovery of activity after exposure of the atria to the drug. In Fig. 1 *A*, the changes in conduction velocity observed when $15\mu\text{g}$ ACh (10^{-6} peak concentration) was added to the bath have been plotted, together with the changes occurring simultaneously in the frequency of the beat. There was no change in the site of the pace-maker, but during the phase of recovery there was a sudden shift in the conduction pathway (Fig. 1 *B*) for a brief period, shown as a gap in the curves in fig. 1 *A*. The second action potential still followed the pace-maker in a regular manner, but at a much shorter interval, illustrating a brief phase of saltatory conduction.

Increases in conduction velocity were observed in every experiment in which the auricles were exposed to sufficient ACh to reduce the contractions by half or more. At low concentrations of ACh, less than 10^{-6} , the increase in

conduction velocity was frequently large, but in some experiments low concentrations produced changes only on the borderline of significance.

Effect of frequency

When atria were driven electrically up to frequencies of 300/sec, it was found that there was a linear inverse relation between frequency and conduction velocity. In Fig. 2 are shown the results of experiments in three preparations illustrating this point at three different temperatures. At higher frequencies the conduction velocity fell off very steeply. If the frequency was still further increased the region under the second electrode failed to respond altogether.

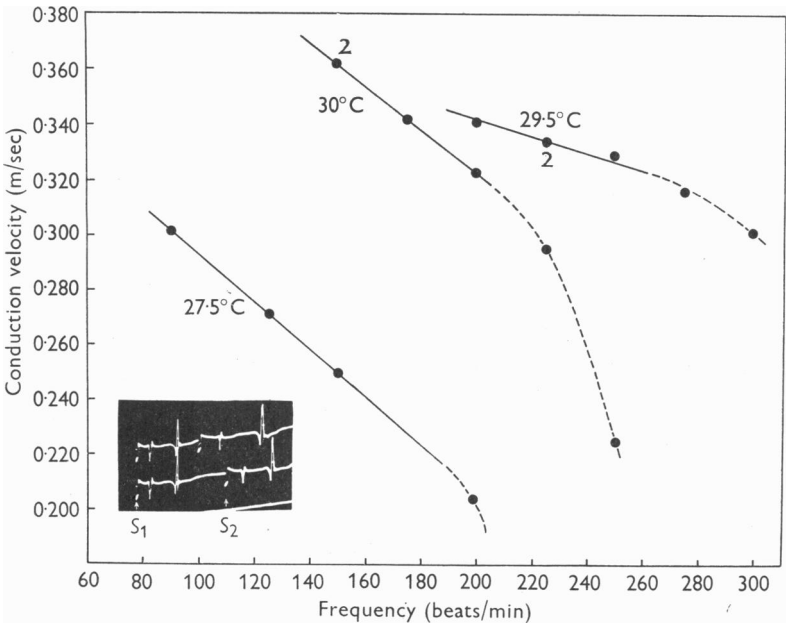


Fig. 2. Relation between conduction velocity and frequency of stimulus at different temperatures, in three auricles. At the points marked 2 the measurements were repeated at the end of the run and found to be the same. Inset: experiment showing that conduction velocity was reduced when the atria responded to a stimulus falling in the relative refractory period. S_1 and S_2 , stimulus artifacts. Each stimulus artifact was followed by action potentials recorded from external electrodes on the right and left atria. The atria were driven at a constant rate (S_1) and a second stimulus was administered outside the relative refractory period (lower trace), and then within the relative refractory period (upper trace), whereupon the interval between the action potentials was prolonged.

The very much steeper reduction in conduction velocity (Fig. 2) at higher frequencies can be explained by the encroachment of the stimulus on the refractory period, since conduction velocity is much slower when the stimulus falls in the refractory period (Fig. 2, inset).

The linear relation between frequency and conduction velocity observed in the driven preparation suggested that the increases in conduction velocity seen after ACh in spontaneously beating auricles might be a consequence of the simultaneous slowing of the rate. The slowing cannot, however, entirely account for the changes in conduction velocity for three reasons. (1) When the changes in conduction velocity and rate were followed from beat to beat it was found that they were not correlated in time. (2) Sometimes there was an early phase of *decreased* conduction velocity (see below) when the rate was already slower.

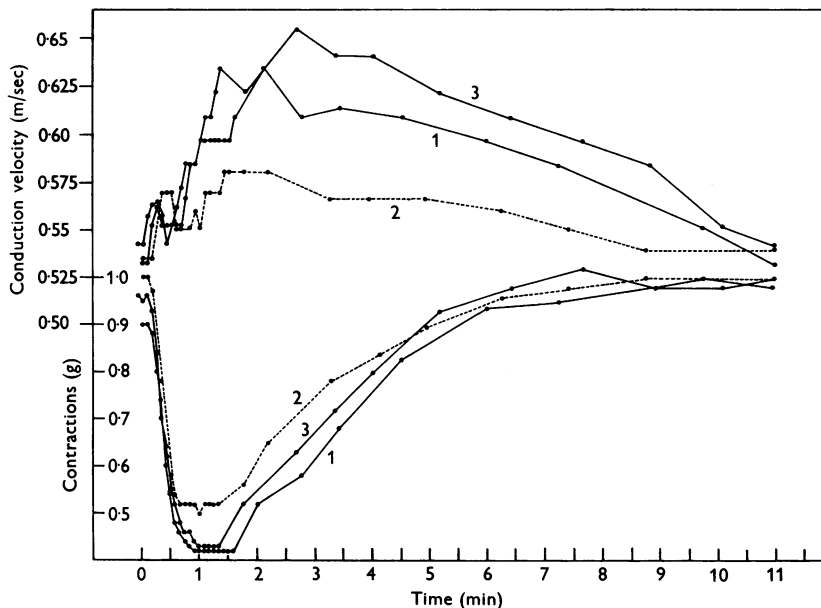


Fig. 3. Simultaneous records of effect of ACh on conduction velocity (upper records) and contractions (lower records). Solid lines, spontaneously beating auricles; broken lines, driven auricle. The experiments were carried out in the order 1, 2, 3.

(3) Some increase in conduction velocity still occurred in the presence of ACh when the atria were driven at a constant rate. The increase was, however, always less than that observed in the spontaneously beating auricle (Fig. 3). In contrast to the effects of frequency on conduction velocity, the reduction in the size of contractions by ACh was not very different in the driven and spontaneously beating preparations (Fig. 3). This would seem to differ from the conclusions of Vane (1957).

If sufficient ACh was given to stop the pace-maker, the conduction velocity during the first beat of recovery was always faster (Fig. 4B). If, instead of adding to the bath a single amount of ACh which was rapidly washed away, the drug was added to the reservoir of solution so that the preparation was

bathed in a more stable concentration, the conduction velocity increased as before, and remained high until the solution was changed (Fig. 4C). The increase in conduction velocity was not, therefore, merely a phenomenon associated with the phase of recovery.

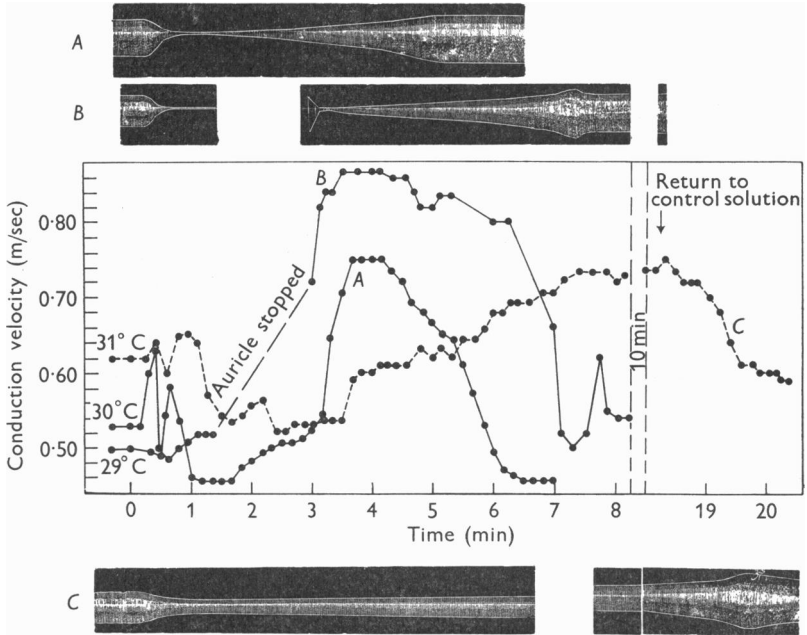


Fig. 4. Simultaneous record of the effect of ACh on conduction velocity and contractions. In *A*, ACh $15\mu\text{g}$ added to the bath slowed the atria. In *B*, ACh $80\mu\text{g}$ stopped the pace-maker for more than a minute, after which the first contraction was large, and conduction velocity fast. In *C* (broken line) ACh 10^{-6} was added to the reservoir of fluid flowing past the auricle, and the increased conduction velocity persisted. In this preparation the increase in conduction velocity was in each case interrupted by a temporary phase of decreased velocity. The graphs have been plotted on the same time scale as the drum speed.

Phase of decreased conduction velocity

In most preparations conduction velocity began to increase immediately after the addition of ACh, and remained fast until the ACh was washed out, as in Fig. 3. In eight atria, however, conduction velocity began to increase, but the increase was then interrupted by a brief phase of decreased velocity, as shown in Fig. 4. This phase always came to an end soon after the peak effect of ACh was established, and gave place to an increase in velocity which thereafter followed the usual time course. Conditions which would either favour or abolish this phase of decreased velocity did not come to light, but a suggested explanation for the phenomenon is given below.

As a result of the above experiments it was concluded that the chief effect of ACh upon conduction velocity in isolated rabbit atria was to produce a pro-

longed increase in velocity, though in some preparations this might be interrupted by a short-lasting phase of decreased velocity. A faster conduction velocity might be the consequence of a swifter rate of rise of the upstroke of the intracellular action potential to the same height, or of an increase in the absolute height of the potential in the same time. In the presence of ACh an increase of more than 10% in the height of the externally recorded action potential was observed in nineteen atria. In three preparations, in the presence of a sufficient concentration of ACh to stop the pace-maker, the height of the action potential was decreased. In the remainder there were no changes in the height of the potential large enough to be regarded as significant. From external records alone, however, it was impossible to decide whether the increase in the action potential height represented a true increase in the magnitude of the intracellular action potential, or was merely the consequence of changes in conduction velocity causing an apparent increase, by reducing the dispersion in time of the arrival of activity in individual fibres under the electrode.

Intracellular records. Simultaneous records of conduction velocity and intracellular potentials were obtained before, during and after exposure to various concentrations of ACh in twenty-two experiments in seven atria. The peak concentrations of ACh varied from 3×10^{-7} to 10^{-4} . In six of the experiments the atria were driven electrically; in the remainder they were allowed to beat spontaneously, so that several records were obtained during the first few beats during the phase of recovery after the pace-maker had stopped.

Measurements made during the period when ACh was exerting its maximum effect (as judged by the reduction in pace-maker frequency and size of contraction, i.e. 1.5–3.5 min after the introduction of ACh into the bath) showed invariably the following effects: (a) an increase in the absolute height of the intracellular action potential; (b) an increase in the rate of rise of the upstroke of the intracellular action potential; (c) an increase in conduction velocity. With one exception, in which the concentration of ACh was 10^{-6} , there was also (d) an increase in resting potential. Photographs of intracellular potentials on a fast sweep, together with simultaneous records of conduction velocity, taken from two experiments before and during the action of ACh, are shown in Fig. 5.

The upstroke of the intracellular action potential is S-shaped, and can be divided into three portions. A slow foot (*AB* in Fig. 6I), followed by a fast linear section *BC*, and ending with a section slowly rising to the summit *CD*. The maximum rate of rise was obtained from the tangent to *BC*, and in the case illustrated was 60 V/sec. The mean rate of rise could be taken as the vertical distance *AD*, divided by the horizontal distance *AD*, and was 30 V/sec. It was found experimentally that these two statistics varied independently. For example, in the initial phase immediately after exposure to ACh the peak voltage was reached earlier, mainly as a result of a shortening of the third terminal section *CD*, without any change necessarily taking place in the

maximum rate of rise, in *BC*. In Fig. 6 II, as an illustration, the mean rate of rise was 35 V/sec, while the maximum rate of rise has been decreased to 48 V/sec. The distinction was not without importance because there is evidence, in Purkinje tissue at least (Weidmann, 1955), that the maximum rate of rise reflects the availability of 'sodium carrier'. An increase in conduction

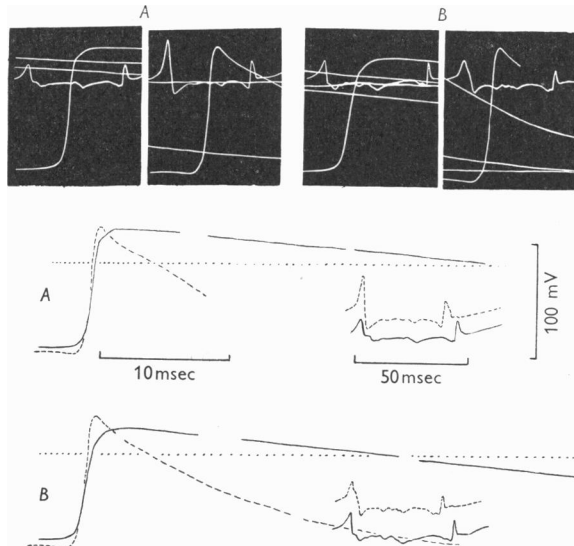


Fig. 5. Simultaneous records of conduction velocity and intracellular potentials in spontaneously beating atria. —, normal beat; ---, at the peak of the effect of ACh $50\mu\text{g}$ added to the bath. The tracings have been made from the photographic records shown above.

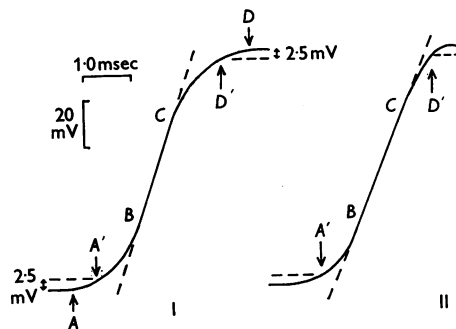


Fig. 6. Diagram of parameters measured. The maximum rate of rise of the upstroke of the intracellular action potential was calculated from the tangent to *BC*. The mean rate of rise was taken as $\frac{(AD - 5)}{A'D'}$ mV/msec, where *A'* and *D'* were points at which the potential crossed a line 2.5 mV above and below its most negative and positive values respectively. These two statistics varied independently. In II the maximum rate of rise was slower than in I, but the mean rate of rise was faster.

velocity could be produced by an increase in the *mean* rate of rise of the intracellular action potential, without necessarily any change occurring in the slope of the steep middle portion of the spike, and it was of interest to know whether there was in fact any increase in the maximum rate of rise.

In practice the exact beginning and end of each upstroke was difficult to estimate. To calculate the mean rate of rise, therefore, the horizontal distance in milliseconds was measured between the points A' and D' (Fig. 6I) at which the rising curve cut a horizontal line 2.5 mV above the most negative value and 2.5 mV below the most positive value of the potential respectively. The mean rate of rise was thus calculated as $\frac{(AD-5)}{A'D'}$ mV/msec.

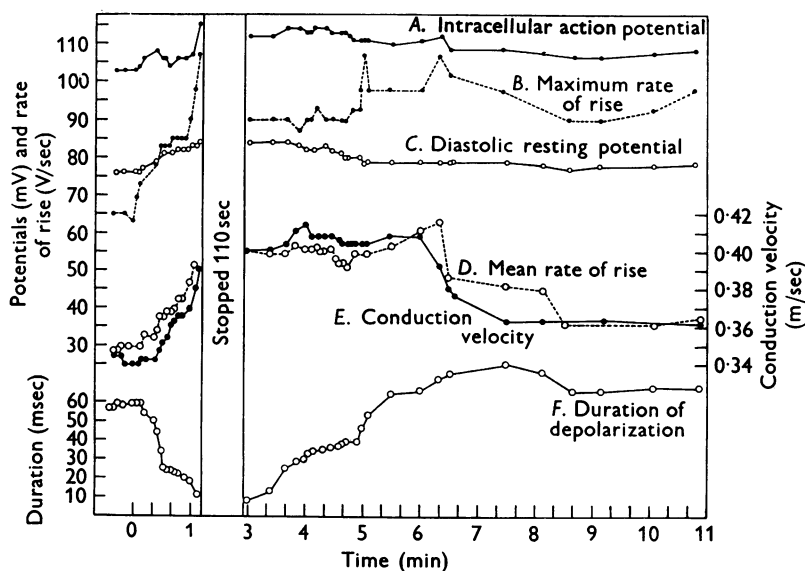


Fig. 7. Parameters of intracellular potential from a single fibre before, during and after the action of ACh. Temp. 31° C. Ordinate: top left, the same numerals apply to millivolts (curves *A* and *C*) and V/sec (curves *B* and *D*); bottom left, msec (curve *F*, duration of action potential at half the height); right, m/sec (curve *E*, conduction velocity). Conduction velocity is correlated with the mean rate of rise.

In Fig. 7 are presented the results of an experiment in which a micro-electrode stayed in a single fibre for more than an hour without a fall in the resting potential. The graph records resting and action potentials, the mean and maximum rates of rise of the upstroke of the action potential, the width of the action potential in milliseconds at half its height, and conduction velocity. It is clear that the duration of the potential bore no relation to conduction velocity. This was a general finding in all the experiments, and is evidence against the suggestion of Ramos & Rosenblueth (1947) that the

duration of the action potential influences conduction velocity. The correlation between conduction velocity and the mean rate of rise is surprisingly close in view of the fact that the potential is recorded from a single fibre, while the conduction velocity represents the average activity of thousands of fibres.

TABLE 1. The effects of ACh on conduction velocity, with simultaneous records of changes in the intracellular resting and action potentials, and in the rate of rise of the upstroke of the latter

ACh concn.	Change in conduction velocity (%)	Resting potential		Action potential		Rate of rise			
		Before (mV)	At peak (mV)	Before (mV)	At peak (mV)	Max.		Mean	
						Before (V/sec)	At peak (V/sec)	Before (V/sec)	At peak (V/sec)
1. 3×10^{-7}	+ 6	80	90	91	99	54	85	25	36
2. 10^{-6}	+10	77	81	90	100	53	103	25	38
3. 10^{-6}	+27	74	81	94	99	64	110	40	51
4. 1.3×10^{-5}	+62	75	83.5	94	106.5	64	85	24	54
5. 1.3×10^{-5}	+ 8	76	80	94	104	56	89	26	42
6. 2.6×10^{-5}	+21	79	84	96	115	69	107	28	63

An increase in the maximum rate of rise of the upstroke was also an invariable feature of the action of ACh at the peak of its effect, both in the driven and the spontaneously beating preparations. In Table 1 the effect of various concentrations of ACh is illustrated. In Expts. 1 and 2 the preparations were driven electrically. Expts. 2 and 3 were performed on the same auricle, driven at 120/min, when the conduction velocity was 0.403 m/sec, and beating spontaneously at 85/min, when the velocity rose to 0.52 m/sec. Although the conduction velocity was initially faster in the spontaneously beating preparation, the same concentration of ACh (10^{-6}) still produced a much greater relative effect on conduction velocity than in the driven preparation, in accordance with the results already described.

From the evidence so far presented the conclusion has been drawn that a true increase in conduction velocity was produced by ACh, associated with an augmentation of the absolute height of the action potential, and of the mean and maximum rates of rise of the upstroke of the potential. Attention may now be turned to seeking an explanation for the phase of reduced conduction velocity sometimes seen during the initial period of exposure to ACh.

Phase of decreased conduction velocity

This was not due to an early reduction in the rate of rise of the upstroke of the active potential preceding the increase described above. A phase of decreased velocity was observed in six of the twenty-two experiments in which simultaneous intracellular potential records were obtained. In every case the rate of rise of the upstroke was increased during the phase of decreased velocity. In ten of the twenty-two experiments there was an initial fall in the height of the action potential, of 3, 3, 3, 3, 4, 5, 8, 8, 8 and 14 mV. In every experiment

the height subsequently increased. The temporary reductions cannot have been the sole factor responsible for the phase of reduced velocity, however, because in six of them (including two when the height was reduced by 8 mV) there was no phase of reduced velocity. In these experiments presumably the concomitant increase in the rate of rise of the upstroke more than counterbalanced the fall in the absolute height. Further, in two experiments, the phase of slower conduction velocity still occurred when the height of the action potential was not reduced. One of these two experiments is illustrated in Fig. 8. The increase in resting potential preceded the increase in action potential, and the conduction velocity was reduced. When the action potential itself increased, then the trend of the conduction velocity reversed, and it, too, was increased.

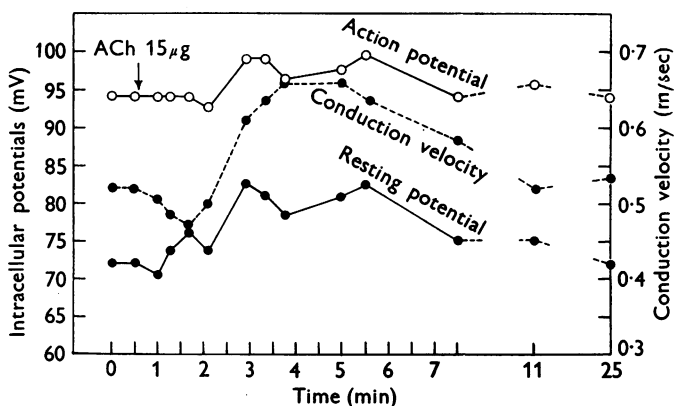


Fig. 8. Record of intracellular potentials during a phase of reduced conduction velocity. The increase in resting potential preceded the increase in the action potential, and the interval corresponded approximately with the phase of reduced conduction velocity.

Detailed examination of all the simultaneous measurements of potential, rates of rise, and conduction velocity has led to the following conclusions. (a) The first observable changes in the shape of the intracellularly recorded potential after exposure to ACh were a shortening of its duration, which had no influence on conduction velocity, and a shortening of the third upper segment of the upstroke of the action potential, leading to an earlier peak and an increase in the mean rate of rise of the upstroke—this tended to increase conduction velocity. (b) The next changes were an increase in resting potential and an increase in the maximum rate of rise of the upstroke, perhaps implying an increased availability of sodium carrier. (c) The absolute height of the action potential was increased, but this increase was sometimes preceded by a temporary decrease. If the decrease was large, or if the increase in resting potential occurred before that of the action potential, there was sometimes a temporary fall in conduction velocity, in spite of the fact that the rate of rise of the upstroke of the action potential was always faster.

The effects of changes in temperature

The above conclusions were open to the criticism that at 30–31° C the resting potential might have fallen so low that the preparation was ‘unphysiological’, and that at a higher temperature the resting potential might be closer to the equilibrium potential for K, and so be unable to increase. Fig. 9 presents the results of five sets of observations, carried out in the order 1–5, at different temperatures. In order to avoid the complicating factor of the changes in the frequency of the pace-maker which would have occurred if the preparation had been permitted to beat spontaneously, the atria were driven throughout at 175 beats/min, a rate which proved faster than the natural pace-maker at 35.5° C, yet not too fast for the auricle to follow at 26.5° C. There was thus no change in frequency throughout the series. Each group of observations con-

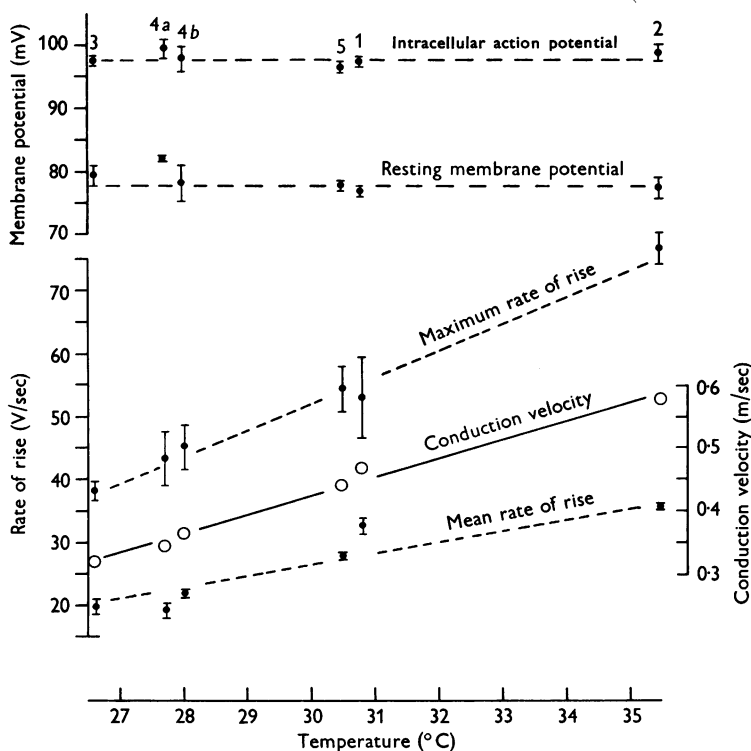


Fig. 9. The effect of temperature upon conduction velocity and intracellular potential. Five sets, of six observations each, were carried out in the order 1–5. Group 4 has been divided into two groups of three because the temperature drifted 0.3° C during the observations. There was no significant change in resting or intracellular action potentials, but the conduction velocity and the mean and maximum rates of rise of the action potential had a Q_{10} of 2. s.e. indicated for each group of observations. The measurements of conduction velocity were almost identical within each group.

sisted of six separate entries. When the temperature was changed the preparation was left half an hour at the new temperature before the observations were made. (Group 4 was divided into two, because the temperature had crept up 0.3° C during the observations.) It is evident that over the range 26.5–35.5° C there was no significant change in the resting or action potentials. The conduction velocity, on the other hand, and the mean and maximum rates of rise of the upstroke of the action potential, had a Q_{10} of about 2. In another preparation, in which simultaneous measurements of conduction velocity and intracellular potentials were made during exposure to ACh at 35° C, the usual increases occurred in the resting and action potentials, in the conduction velocity, and in the mean and maximum rates of rise of the upstroke.

The effect of adrenaline

It has been reported that in the frog sinus (Hutter & Trautwein, 1956) sympathetic stimulation caused an increase in the rate of rise and in the absolute height of the action potential. There is also evidence (Hoffmann, Hoffmann, Middleton & Talesnik, 1945; McDowall, 1946) that ACh can excite post-ganglionic sympathetic neurones. The possibility had to be considered, therefore, that the effects of ACh on conduction velocity and on the shape of the intracellular potential described above were not the true effects of ACh itself, but secondary to a simultaneous stimulation of post-ganglionic sympathetic nerves. In several experiments, however, one of which is illustrated in Fig. 10, it was found that moderate amounts of adrenaline, sufficient to increase the force of contraction by 50–100 %, had no effect on conduction velocity, nor on the mean and maximum rates of rise of the intracellular action potential. Higher concentrations decreased the conduction velocity and the rate of rise of the action potential. The results of two other experiments are given in Table 2. In both these experiments the atria were driven at 175 beats/min, a rate which proved fast enough to prevent the natural pace-maker from taking over even in the presence of adrenaline. In both experiments there was an increase in the absolute height of the action potential.

It was shown recently by Bertler, Carlsson & Rosengren (1956) that the injection of reserpine 5 mg/kg intravenously into rabbits was followed within 16 hr by a virtually complete removal of catechol amines from their hearts. This observation made it possible to test in another way whether the excitation of post-ganglionic fibres could be involved in the action of ACh, since a preparation could be obtained from which all sympathetic transmitter had been removed. Accordingly, a rabbit was injected intraperitoneally with 0.5 mg/kg of reserpine, and after 24 hr intravenously with 5 mg/kg of reserpine. This procedure has been shown to reduce the catechol amine content of auricles to less than 0.3 % of their original value. Sixteen hours after the second injection

of reserpine the atria were set up, and simultaneous measurements of conduction velocity and intracellular potential were made during exposure to various concentrations of ACh. The results did not differ (Table 2) from those obtained in the other experiments. It has been concluded, therefore, that the changes in conduction velocity and intracellular potential reported above were the consequence of the effects of ACh, and not secondary to a simultaneous stimulation of post-ganglionic sympathetic fibres.

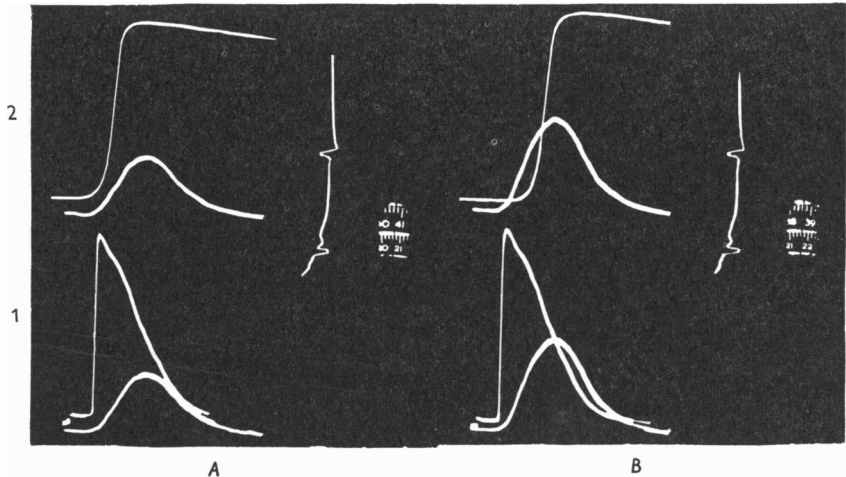


Fig. 10. The effect of adrenaline. Photographs of intracellular potential and contraction during successive beats (1 and 2). The lower trace in each frame is the transducer record of contraction. Immediately above is the intracellular potential record. The latter has been photographed at slow and fast sweep speeds (frames 1 and 2). On the right of frame 2 the vertical trace shows action potentials recorded from two external electrodes, and on the extreme right the time at which each photograph was taken is recorded to within 0.1 sec. The preparation followed electrical stimuli throughout at 175/min. *A*, normal beats (at 20 min 40.7 sec); action potential height, 94 mV. *B*, in the presence of adrenaline 0.3 µg/ml. (at 21 min 38.7 sec); the action potential was now 97 mV and the contraction had increased by 50%; the conduction velocity was unchanged. The apparent increase in the rate of rise of the upstroke is an illusion due to the increased height.

TABLE 2. Comparison of the effect of adrenaline with the effect of ACh on a preparation from which sympathetic transmitter had been removed by previous injections of reserpine

Drug	Change in conduction velocity (%)	Resting potential (mV)		Action potential (mV)		Mean rate of rise (V/sec)		Max. rate of rise (V/sec)		Change in contraction (%)
		Be-fore	At peak	Be-fore	At peak	Be-fore	At peak	Be-fore	At peak	
Adr 3×10^{-7}	0	77.5	77.5	96	103	35	35	68	68	+ 60
Adr 1.3×10^{-6}	0	79	79	90	94	29.8	28	61.5	42	+240
Atria from rabbit previously injected with reserpine										
ACh 10^{-6}	+10	78	81	88	99	28	32	64	100	-40
ACh 10^{-4}	+57	76	83	87	97	24	54	56	110	-79

DISCUSSION

Previous evidence concerning the effect of vagal stimulation or of ACh on conduction velocity in mammalian atrial muscle has led to conflicting conclusions. Lewis *et al.* (1920) found that vagal stimulation caused no change in conduction velocity in dog atria, either when beating spontaneously or when driven electrically at a constant frequency. Brooks *et al.* (1956), on the other hand, also working with dogs, observed that conduction velocity was increased by vagal stimulation, but Burgen & Terroux (1953*b*) found that ACh and carbamylcholine reduced conduction velocity in isolated strips of atria taken from cats. Webb & Hollander (1956) found that the action potential in rat atria was about 70 mV and was slightly increased by ACh. They observed that ACh reduced conduction velocity but did not cause any fall in the height of the intracellular action potential. In the present series of experiments with isolated rabbit atria, it was found that the main effect of ACh was to increase conduction velocity, though in some auricles the increase was interrupted by a temporary phase of decreased velocity when the concentration of ACh was high. Simultaneous records of conduction velocity and intracellular potential have provided an explanation for these effects.

ACh caused an increase in the resting potential and in the rate of repolarization in the rabbit atrium, as has already been reported in the cat and dog. At the peak of the effect of ACh there was an increase both in the absolute height and in the rate of rise of the intracellular action potential. These observations provided an adequate explanation for the increased conduction velocity. The increase in the rate of rise of the upstroke of the action potential was due not merely to a shortening of the slower terminal portion of the spike, but there was also a steepening of the fast central part, implying an increased rate of entry of sodium.

The increases in conduction velocity developed gradually and followed a characteristic course. Sudden changes in the interval between the recorded action potentials did also occur, but were easily recognized as shifts in the pathway of conduction or occasionally in the site of the pace-maker. The externally recorded action potentials frequently increased in size in the presence of ACh, and in view of the results of the intracellular recordings it is likely that these changes represented true increases in height of the intracellular action potential, and not just a reduction in the dispersal of individual potentials caused by conduction velocity changes.

When the atria were stimulated electrically it was found that there was an inverse linear relation between conduction velocity and the frequency of stimulation. The increase in conduction velocity in the presence of ACh was not entirely due to the concomitant slowing of the pace-maker, however, because increases still occurred when the atria were driven at a constant

frequency, though the increases were less than in spontaneously beating preparations.

The finding of an inverse relation between frequency and conduction appears to differ to some extent from previous evidence. Prinzmetal, Corday, Brill, Oblath & Kruger (1952) did observe such a relation in dog atria at stimulation frequencies above 350/min, but at lower frequencies the stimulus-potential interval was unchanged. The stimulus-potential interval, however, cannot always be assumed to measure conduction velocity, because changes in the latter might be concealed by opposite changes in latency. Brooks *et al.* (1956) stated that in dog atria there was no change in conduction velocity until the stimulation frequency reached 250/min. The evidence they presented (Fig. 4C, p. 196) did show, nevertheless, in two of the three curves illustrated, a slight slope, correlating frequency inversely with conduction velocity, at frequencies between 100 and 240/min. It is possible that the upstroke of the action potential involves a reaction requiring a reagent drawn from a source common to the mechanism of contraction. When contractions are more frequent a reduction in the source might be revealed as a slowing of the upstroke. Such a phenomenon would be likely to occur at lower frequencies in an isolated preparation than in the better oxygenated whole atrium supplied with blood.

In some auricles high concentrations of ACh produced a temporary fall in conduction velocity before the usual increase. Simultaneous recordings of conduction velocity and intracellular potential showed that this phase of reduced velocity was not due to any slowing of the rate of rise of the upstroke of the action potential, which was invariably faster in the presence of ACh. Hutter & Trautwein (1956) explained the decrease of conduction velocity which they observed after vagal stimulation in the frog sinus as due to a fall in the height of the action potential. So far as the phase of reduced conduction velocity in mammalian atria is concerned, a fall in the height of the action potential cannot account for all the facts. Burgen & Terroux (1953*b*) observed that carbamylcholine always reduced conduction velocity in cat auricle strips, but they stated 'the magnitude of the active membrane potential was unchanged except with the highest concentrations used (50–100 $\mu\text{g/ml.}$), where some of the action potentials were reduced.' In the present experiments a temporary reduction in the height of the action potential was observed on six occasions when the conduction velocity was not reduced and even increased, and on two occasions conduction velocity was reduced when the height of the action potential was not. In these latter cases it was noted that the increase in resting potential slightly preceded the increase in height of the action potential. It has been concluded that conduction velocity is influenced not only by the rate of rise and the height of the action potential, but also by the time at which the increases in these parameters occur relatively to the increase in resting potential.

The absolute height of the action potential was always increased by ACh at the peak of its effect. Burgen & Terroux (1953*b*) never observed an increased 'overshoot' in cat atria, and they stated that 'the rate of rise is little affected', though some increase in the rate of rise is indicated in their Table 2. Hoffman & Suckling (1953) also found an increased rate of rise of the action potential in dog atria after vagal stimulation. The rates of rise in rabbit atria were approximately five times as fast as those reported for the cat by Burgen & Terroux (1953*a*).

Hutter & Trautwein (1956) (in experiments in which the pace-maker frequency was free to increase) observed that both the rate of rise and the height of the action potential were augmented by *sympathetic* stimulation in the frog sinus, and the possibility had to be considered that the results described here were due not to ACh itself, but to stimulation by ACh of post-ganglionic sympathetic neurones. In the experiments described above the frequency of contractions was kept constant, and in these circumstances it was found that adrenaline, although increasing the height of the action potential slightly, did not change the conduction velocity or the rate of rise of the upstroke, and higher concentrations actually reduced them. Further, the usual increases in conduction velocity and rate of rise after exposure to ACh still occurred in atria from which all sympathetic transmitters had been removed by previous treatment with reserpine. It was concluded, therefore, that simultaneous excitation of post-ganglionic sympathetic neurones was not involved.

Previous studies of the effects of temperature changes on various cardiac tissues (Woodbury, Hecht & Christopherson (1951) in frog ventricle, Trautwein (1953) and Corabœuf & Weidmann (1954) in mammalian Purkinje fibres, Burgen & Terroux (1953*a*) in cat auricle) concur in concluding that there is little alteration in the resting or intracellular action potential between 25° and 37° C, and the present findings have shown that this is also true of the rabbit atrium. So far as the rate of rise of the upstroke of the action potential is concerned, however, there would appear to be some disagreement with the series of observations of the effects of temperature carried out on mammalian auricle by Burgen & Terroux (1953*a*) who stated 'with fall of temperature the rate of rise of the action potential was affected to only a trivial extent between 38° and 24° C'. In the experiments described here it was observed that between 26.5° and 35.5° C there was no significant change in either the resting or action potentials, but the conduction velocity and the rate of rise of the upstroke both had a Q_{10} of about 2.0. The mechanism responsible for ionic transport during diastole evidently has a big margin of safety, being able to maintain ionic concentration differences at their limiting value over a wide range of temperature. The process preceding or accompanying the flow of ionic current during activity, on the other hand, at any rate in rabbit atria, involved a chemical reaction whose rate was highly temperature-dependent. This would be

consistent with the inverse relation between conduction velocity and frequency of contraction, if the reactions involved in ion transport during membrane activity and in contraction shared a common reagent. Both contraction and conduction velocity are very sensitive to a reduction in the supply of oxygen. The observation that ACh increases the maximum rate of rise of the action potential is thus of interest, as it implies an influence on the availability of sodium carrier, either directly or as a consequence of the increase in resting potential.

A series of papers in the last few years (Burgen & Terroux, 1953*b*; Fatt, 1954; del Castillo & Katz, 1955; Hutter & Trautwein, 1956; Trautwein, Kuffler & Edwards, 1956) has led to the view that the resting potential in atrial muscle is lower than that theoretically calculable from the ratio of the internal and external concentrations of K, and it has been suggested that ACh opens channels in the membrane wide enough to permit a passive efflux of K, while remaining sufficiently narrow to impede the passive influx of Na. The intracellular potential thus becomes more negative, because the less restricted diffusion of K permits the resting potential to approach the value it could theoretically attain if K were at equilibrium. A similar argument may now be advanced in respect of Na. For since, in the presence of ACh, the intracellular action potential may increase from 97 mV (-79 to $+18$) to as much as 115 mV (-84 to $+31$), to take an extreme example, the implication is that during normal activity the 'overshoot' is well below the equilibrium potential for Na.

The considerable literature concerning the effects of vagal stimulation upon auricular arrhythmias has been reviewed by Prinzmetal *et al.* (1952). It was recently shown by Burn, Vaughan Williams & Walker (1955) that in the dog heart-lung preparation atrial fibrillation could be turned on and off at will by a combination of electrical stimulation with the infusion of ACh. It is possible that the increase in the rate of rise of the action potential produced by ACh, representing a greater availability of sodium carrier, is concerned in the phenomenon. If so, the ability of potassium to arrest fibrillation would be readily explained, since the accompanying fall of membrane potential decreased the rate of rise of the upstroke (Weidmann, 1955, 1956), although the phase of repolarization was much more rapid. Burn, Gunning & Walker (1957) found that adrenaline and noradrenaline could antagonize the development of fibrillation. Webb & Hollander (1956) reported that adrenaline prolonged the duration of the intracellularly recorded action potential in rat atria. Although they did not measure the rate of rise of the potential, they observed that conduction velocity was reduced by adrenaline, as found here. It is possible that a reduced availability of sodium carrier is concerned in the antagonism of adrenaline to the production of fibrillation in atrial muscle.

SUMMARY

1. ACh increased conduction velocity in isolated rabbit atria, although in a few preparations the increase was interrupted by a temporary phase of decreased velocity.

2. There was an increase in the rate of rise and absolute height of the intracellular action potential in the presence of ACh, which could account for the increased conduction velocity. There was also an increase in resting potential and rate of repolarization.

3. In some experiments a temporary fall in the height of the intracellular action potential preceded the usual increase. This could not wholly account for the phase of decreased conduction velocity, which sometimes occurred in the absence of a fall in the height of the intracellular action potential. In these cases it was observed that the increase in resting potential preceded that of the intracellular action potential.

4. Evidence was presented that the above effects were truly those of ACh, and not due to stimulation by ACh of post-ganglionic sympathetic neurones.

5. There was an inverse linear relation between conduction velocity and the frequency of contraction.

6. Between 35.5° and 26.6° C there was no significant change in the resting or intracellular action potentials. Conduction velocity, however, and the rate of rise of the intracellular action potential had a Q_{10} of about 2.

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