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PACEMAKER POTENTIALS. THE EXCITATION OF ISOLATED RABBIT AURICLES BY ACETYLCHOLINE AT LOW TEMPERATURES

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In the course of a recent investigation (Vaughan Williams, 1955) in which, *inter alia*, a few measurements were made of the Q_{10} of conduction velocity in isolated rabbit auricles, some of the auricles stopped beating at the lower end of the temperature range being studied. It was noticed on two occasions that although all mechanical activity had apparently ceased, small regularly occurring potentials continued in the region of the pacemaker. This phenomenon has now been studied in more detail. The effects of temperature changes on the behaviour of various preparations of isolated cardiac muscle have been examined before (Knowlton & Starling, 1912; Clark, 1920; Burgen & Terroux, 1953*a*; Trautwein & Gottstein, 1953; Trautwein, Gottstein & Federschmidt, 1953; Coraboeuf & Weidmann, 1954). The use of cooling in the present experiment is, therefore, not of particular interest, except as a means of separating the activity of the pacemaker region from that of the rest of the auricle. It has been possible to show that the link thus broken can be re-established by acetylcholine. An 'excitatory' action of acetylcholine has previously been demonstrated by several authors (Spadolini & Domini, 1940; Bülbring & Burn, 1949; Spadolini & Giachetti, 1953; Holtz & Westermann, 1955).

METHODS

Rabbit auricles were dissected and mounted in a Perspex chamber (Vaughan Williams, 1955). For the cooling experiments any desired temperature below room temperature could be maintained by a device, controlled by a thermostat, which at intervals directed the water circulating in the water-jackets through a heat-exchanger standing in iced water. The combination of this with the thermostatically controlled heater already described permitted the temperature to be held constant at any value between 10 and 35° C.

The solutions which flowed continuously through the auricle chamber were 308 milliosmolar; Na^+, 139·7; K^+, 5·63; Ca^{2+}, 2·165; glucose, 11·12; Cl^- and HCO_3^- together, 149·66. The bicarbonate

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concentration was adjusted to give a pH of 7·4 with the gas mixture used, which was usually 2% CO_2, 98% O_2.

Electrical records from the surface of the auricle were taken with two bipolar electrodes leading to two independent preamplifiers, whose outputs were displayed on the two beams of a Dumont 322 oscilloscope. Contractions were recorded almost isotonically, since the auricle pulled a long lever attached to an RCA 5734 transducer, whose output was displayed on another oscilloscope. Traces from both oscilloscopes were photographed simultaneously on the same film (Grass camera, model C4C), together with a record of the time. The maximum sensitivity of the tension recorder in most experiments was 20 mg for a 2·5 cm deflexion of the oscilloscope beam.

The acetylcholine chloride solutions were made up immediately before use from ampoules (Roche).

RESULTS

Auricles were set up in the recording chamber, and one electrode was placed in position to record an action potential from the left auricle. The right auricle was then explored with the second electrode until the interval in time between the two action potentials was maximal; the second electrode was then on or near the pacemaker. The auricle was allowed to beat at 30° C without disturbance for 90 min, since previous experience had shown that at the end of this time auricular activity reached a plateau of maximum stability in the rate and force of the beat, and in conduction velocity.

TABLE 1. A summary of the measurements of rate and conduction velocity and their corresponding values for Q_{10} at 30, 25 and 20° C

° C	Mean	Range of values	No. of observations	S.D. about mean	S.E. of mean	° C	Mean Q_{10}	Range of Q_{10} values	No. of observations	S.D. about mean	S.E. of mean
					A. Rate (beats/min)						
30	90	140–60	25	16	±3·3	30–25	2·8	3·9–1·6	19	0·49	±0·11
25	61	80–45	19	11	±2·5	25–20	2·5	5·0–1·8	18	1·0	±0·24
20	33	58–10	18	13	±3·0	—	—	—	—	—	—
					B. Conduction velocity (m/sec)						
30	0·643	1·42 –0·323	24	0·247	±0·050	30–35	2·7	3·7–1·8	19	0·530	±0·120
25	0·441	1·00 –0·190	19	0·200	±0·046	25–20	3·8	6·0–2·3	15	1·280	±0·330
20	0·238	0·600–0·074	15	0·131	±0·034	—	—	—	—	—	—

Effects of cooling. After the initial control period at 30° C, the auricle was slowly cooled down until it stopped. The recorded amplitude of contraction at first increased as the speed of its development became slower, but decreased when the temperature fell below 22° C. The rate of the beat and the conduction velocity both steadily diminished. The mean values for rate and conduction velocity over the temperature range 20–30° C, together with figures for Q_{10}, are given in Table 1. Below 20° the activity of most of the auricles became less regular, so that measurements of rate and conduction velocity varied more widely. As cooling continued, the end-point for the cessation of all measureable electrical and mechanical activity was quite sharp, and was characteristic for each auricle. All auricles stopped at some temperature between 20 and

14° C. When an auricle was cooled a second or a third time it was usually found that activity persisted to a temperature at least half a degree lower than that at which it had ceased on the previous occasion. This phenomenon is illustrated in Fig. 1.

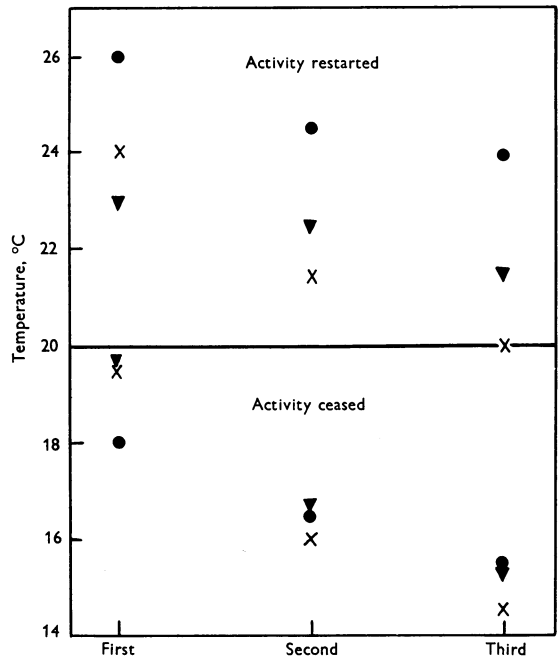

Fig. 1. Effect of repeated cooling and rewarming. Top: temperatures at which activity restarted in three auricles when each was warmed on three successive occasions from a temperature at which all activity had ceased. Bottom: temperature at which all activity ceased on repeated cooling.

Pacemaker potentials. As the temperature was lowered, the action potential diminished and disappeared from the left auricle, while contractions in the right auricle persisted. The wave of activation travelled less and less far from the region of the pacemaker, until finally no further tension could be measured. At this time, however, potentials could still be recorded from the region of the pacemaker. Very small, regularly occurring potentials persisted to a temperature two or three degrees below that at which contractions ceased to be measurable. On further cooling these small potentials, too, disappeared.

The actual range of temperatures over which these pacemaker potentials only were seen, varied from one auricle to the next. Fig. 2 shows simultaneous electrical and mechanical records from three different auricles cooled to temperatures at which only pacemaker potentials remained. No mechanical activity could be recorded at these temperatures, although the sensitivity of

the mechanical recording system was 8 mg/cm on the oscilloscope screen and in some experiments this was further increased to 2·2 mg/cm.

If the auricles were cooled further all activity ceased, but, on rewarming, the pacemaker potentials reappeared first and were confined to a very small area in the right auricle. As the temperature was gradually raised a sharp and definite threshold was reached at which faster potentials made their appearance (Fig. 3), 'taking off', as it were, from the pacemaker potential, and associated with the reappearance of measurable contractions. Once the threshold temperature had been reached, the faster action potentials associated with tension spread progressively outwards from the pacemaker region, although the temperature was not raised further, until after a few beats the auricle once more began to contract as a whole.

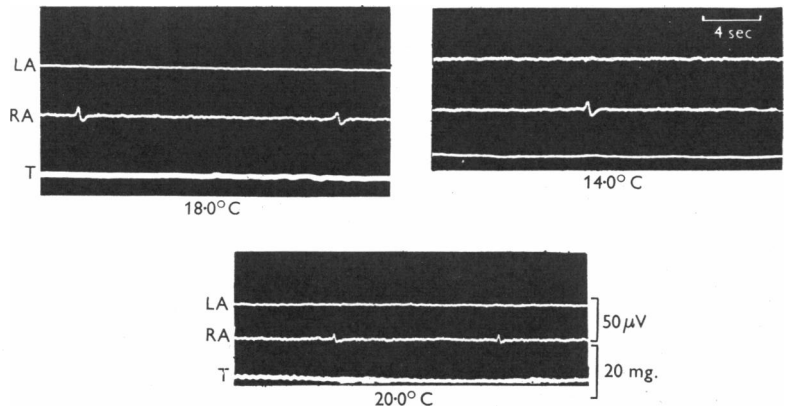

Fig. 2. Pacemaker potentials from three auricles. RA, record from electrode placed on the pacemaker. LA, record from left auricle. T, tension.

The fact that no tension associated with the pacemaker potentials could be measured, even with such a sensitive recorder, does not prove that no movement was present. Some small contractions beyond the reach of our instruments may have persisted in the pacemaker region. The point of interest is that activity continued in this region at temperatures several degrees below that at which it had ceased in the rest of the auricle. When the threshold temperature for the latter was reached, a fast type of action potential made its appearance, which was conducted outwards, and tension was always immediately and easily recorded.

Location of the pacemaker. Potentials in the absence of measurable contractions were seen on cooling in nineteen out of twenty-five experiments. In seventeen of these nineteen experiments the potentials were found in exactly the region which had already been selected as the point of origin of the normal beat at 30° C before cooling, and were immediately lost if the electrode

was moved a few millimetres away. It is for this reason that these small and regularly occurring potentials have been called 'pacemaker potentials'.

In the eighteenth experiment the pacemaker potentials after cooling were found about 3 mm from the position estimated as that of the original pacemaker. In the nineteenth experiment (an early one), small potentials, not associated with measurable tension, were found in both right and left auricles, occurring at different frequencies. When the tissue was warmed, both auricles

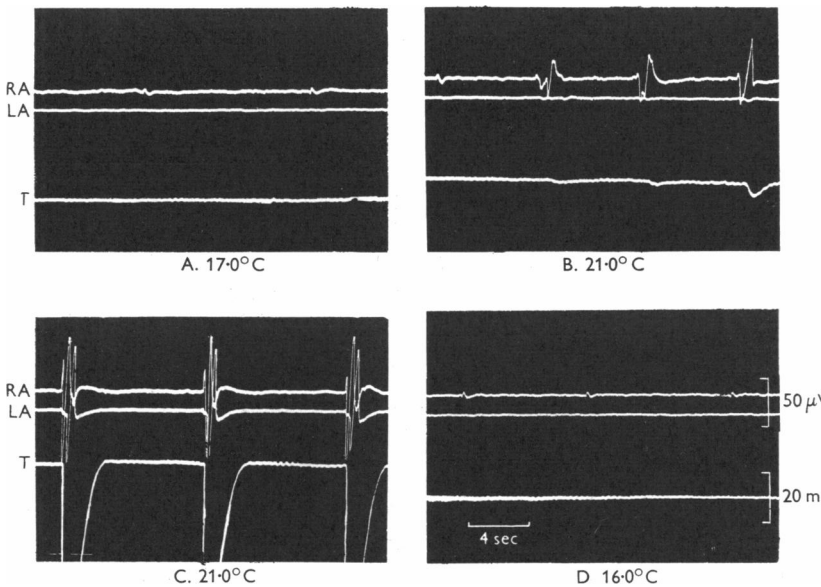

Fig. 3. Effect of cooling, warming and cooling. A, at 17° C, pacemaker potentials only; no tension could be measured. B, auricle warmed, pacemaker potentials persisted until the threshold for firing neighbouring tissue was reached at 21° C. C, temperature still at 21° C; conduction had spread outwards, so that both right and left auricles are contracting. D, auricle cooled again; pacemaker potentials persist at 16° C. Retouched.

followed the pacemaker in the right auricle. Such independent potentials from the left auricle were not seen in any subsequent experiment, and for this reason we are inclined to the view that they may have originated from an ectopic focus or damaged area, rather than that there was a second 'pacemaker' in the left auricle.

Experiments in which no pacemaker potentials were observed. In the remaining six of the twenty-five experiments, all electrical and mechanical activity ceased abruptly when the auricle was cooled. No pacemaker potentials could be found, although both left and right auricles were explored. These experiments are described more fully in the section which follows.

Effects of acetylcholine. In fifteen experiments, when the auricle had been cooled to a temperature at which only pacemaker potentials were seen, acetylcholine was introduced either as a single amount added directly to the recording chamber, or as a constant concentration in the fluid flowing through the bath. In all these experiments there was a latent period of 1 or 2 min in the presence of acetylcholine, during which no consistent change in the behaviour of the auricle was observed. Then, in every experiment, large, fast, propagated action potentials suddenly took off from the pacemaker potentials (Fig. 4B) and large contractions were simultaneously recorded. These continued for some time after the acetylcholine had been washed out of the bath, but after a few minutes the auricle again stopped beating and only the pacemaker potentials remained.

Concentrations of acetylcholine between 10^{-6} and 10^{-8} g/ml. proved to be the most effective. This response to acetylcholine was abolished by adding atropine (10^{-7} g/ml.) to the bath at the height of the ACh effect (Fig. 4D–F). Alternatively, if the tissue was bathed in a solution containing atropine before the

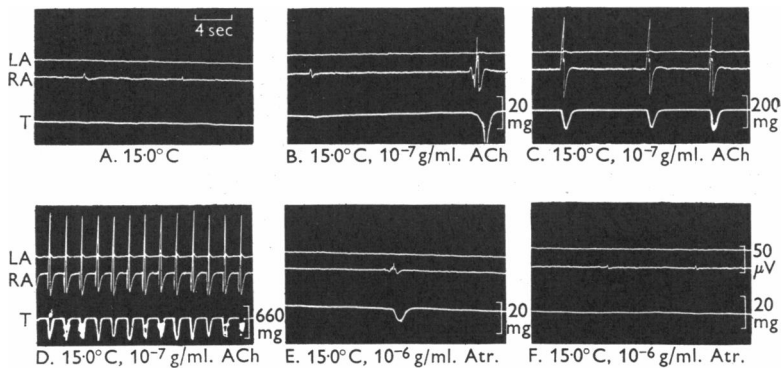

Fig. 4. Effect of acetylcholine and atropine. A, pacemaker potentials only. B, 2 min after the addition of acetylcholine, 10^{-7} g/ml. C, 1 min later. D, 7 min later. E, 5 min after the addition of atropine, 10^{-6} g/ml. F, 10 min later. Temperature 15° C throughout; retouched.

acetylcholine was added, the latter was unable to restart the auricles at the low temperature. Atropine itself had no effect on the pacemaker potentials. Concentrations of acetylcholine lower than 10^{-6} g/ml. had no effect on the spontaneously beating auricles at 30° C.

As stated above, in six experiments both electrical and mechanical activity stopped simultaneously, and no pacemaker potentials could be found. The evidence so far had suggested that in the region of the pacemaker there was some specialized tissue capable of producing regular non-propagated potentials associated with negligible tension, whereas the rest of the auricle responded to these with propagated potentials and easily measured contractions. The link

between these two distinguishable processes was broken by cooling. If, as usually happened on cooling, the second process failed first, the activity of the pacemaker region alone persisted, and the pacemaker potentials were revealed. If, on the other hand, the pacemaker failed first, no activity at all could be detected, because there were no pacemaker potentials to which the rest of the auricle could respond. If this hypothesis were correct, the auricle should still be able to respond to an electrical stimulus. The experiment shown in Fig. 5 supports this view.

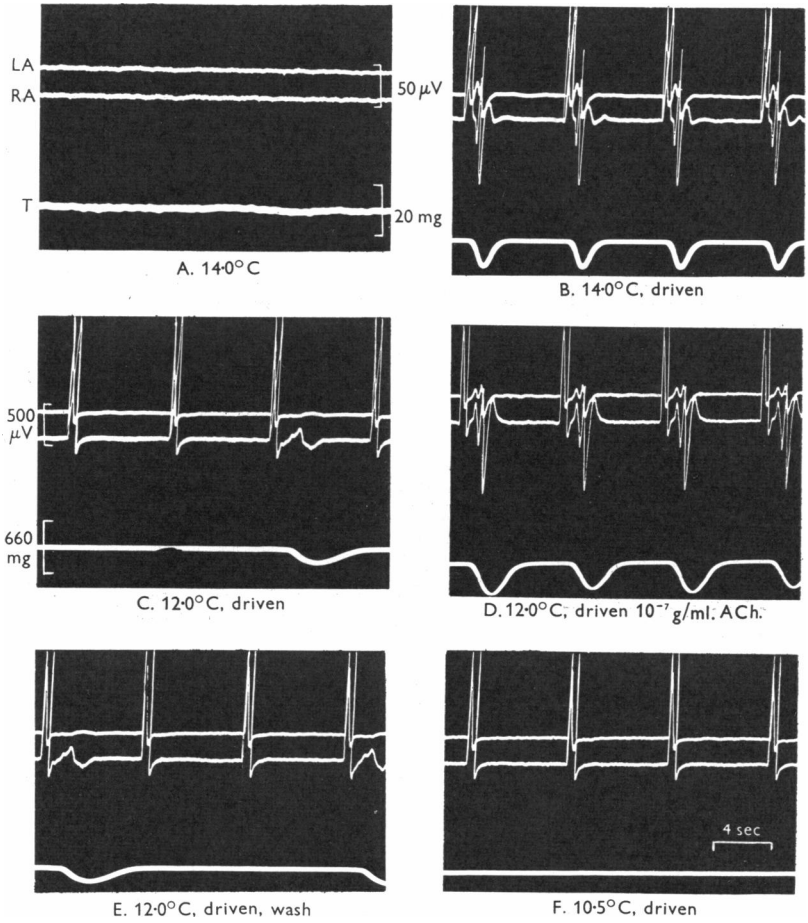

Fig. 5. Electrical stimuli applied to replace pacemaker. A, both electrical and mechanical activity cease abruptly at 14° C. B, electrical stimuli applied; both left and right auricles respond (compare F, in which stimulus artifacts alone appear). C, at 12° C only right auricle responding to every third stimulus. D, in presence of acetylcholine, 10^{-7} g/ml., both auricles respond to every stimulus. E, after acetylcholine washed out, only right auricle responds to every third stimulus. F, at 10·5° C no response; stimulus artifacts only. Retouched records.

In Fig. 5 the auricle had suddenly stopped and no pacemaker potentials could be found, but when stimulated (Fig. 5B) it responded to every stimulus. The auricle was then cooled further, in an attempt to simulate the conditions in the other auricles when the auricle just failed to respond to persisting pacemaker potentials. In Fig. 5C the auricle was responding, on the right side only, to every third stimulus. At this point acetylcholine was added, and soon afterwards the auricle began to respond once more to every stimulus, and the action potentials were conducted to the left side (Fig. 5D). When the acetylcholine had washed through, activity diminished, until the right auricle only was responding once more to every third stimulus (Fig. 5E). On further cooling all activity ceased, and stimulus artifacts only were seen (Fig. 5F). The addition of acetylcholine at this point had no effect, neither did it evoke contractions at a higher temperature in the absence of a stimulus (Fig. 5A). It thus appeared that some initiating stimulus, either in the form of a pacemaker potential or an electric shock, had to be present for the acetylcholine to be effective.

In four experiments the auricles were cooled and rewarmed, first in normal control solution, and then in a solution containing 10^{-6} g/ml. acetylcholine. The temperature at which the pacemaker potentials only were observed was not significantly different in the two solutions. In the acetylcholine solutions, however, the phase during which only the pacemaker potentials were seen was very brief, because the rest of the auricle continued to respond almost until the initiating stimulus stopped. Similarly, when the auricle was rewarmed in the presence of acetylcholine, propagated action potentials appeared very soon after the pacemaker potentials were first seen and at a temperature at which only pacemaker potentials had been present in the control solution.

Effect of eserine. Eserine in concentrations from 10^{-5} to 10^{-7} g/ml. had no effect on the size or frequency of the pacemaker potentials, but the amount of acetylcholine necessary to make the rest of the auricle respond to the pacemaker potentials was 10 to 1000 times less in the presence of eserine. In six experiments eserine was added as a constant concentration to the solution bathing the auricle, for periods up to $1\frac{1}{2}$ hr. In the experiment illustrated in Fig. 6, at 16° C when only pacemaker potentials were visible, acetylcholine 10^{-7} g/ml. initiated an action potential and tension, but the activity was not propagated to the left auricle (Fig. 6B). Acetylcholine 10^{-6} g/ml. achieved the production of an action potential which was propagated to the left auricle (Fig. 6C). When the solution passing through the bath contained 2×10^{-6} g/ml. eserine, however, a concentration of acetylcholine as low as 10^{-9} g/ml. elicited a propagated action potential.

The rate of the pacemaker. The auricular rate below 20° C was less regular than above this temperature. In some experiments the rate of the pacemaker potentials increased after the addition of acetylcholine, and it was at first

thought that this might be a regular effect of acetylcholine. Examination of the whole series of experiments, however, revealed that there was no consistent change in the rate of the pacemaker potentials in the interval (sometimes 2 min) after the addition of acetylcholine, and before the commencement of contractions and propagated action potentials. Once the contractions had started, the rate always increased, at least to some extent (Fig. 4D), and it seemed as though the activity provoked by acetylcholine and not the acetylcholine itself was responsible. In sum, no consistent change in the shape or frequency of the pacemaker potentials themselves was produced by acetylcholine, eserine or atropine. The action of these drugs seemed to be not upon the production of the pacemaker potentials, but upon the effects of the latter on the rest of the auricle.

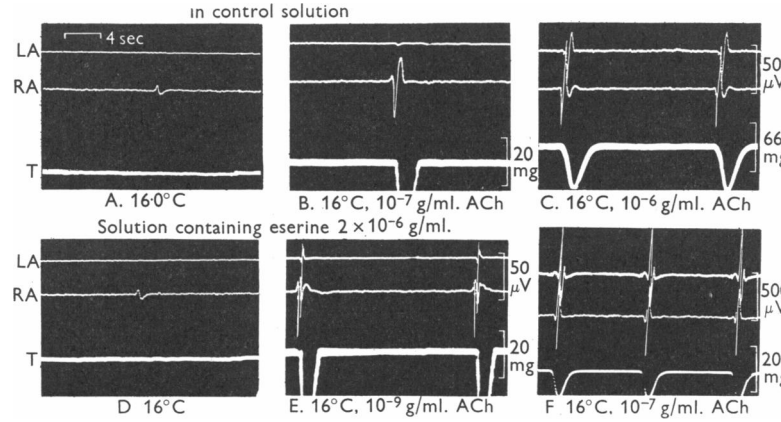

Fig. 6. A–C, auricle in control solution. D–E, auricle in solution containing eserine 2×10^{-6} g/ml. A, pacemaker potentials only. B, effect of ACh 10^{-7} g/ml. C, effect of ACh 10^{-6} g/ml. D, pacemaker potentials only. E, effect of ACh 10^{-9} g/ml. F, effects of ACh 10^{-7} g/ml. Retouched records.

In Fig. 7 the rate of the pacemaker, the height of the action potential, and the force of contractions, have been plotted against time, first when an auricle was warmed, and secondly when the electrical and mechanical activity were restored by the addition of acetylcholine in the same experiment. In Fig. 7A, when the temperature was raised, the pacemaker potentials increased in rate with very little increase in size until a critical temperature was reached at which propagated action potentials appeared simultaneously with measurable tension. Thereafter, the size of the action potential and tension increased together, although the temperature remained unchanged. With acetylcholine, at low temperature, the picture was rather different, as seen in Fig. 7B. When the acetylcholine was added the first thing that happened, after a latency of about 2 min during which time there was no change in either the rate or amplitude of the pacemaker potentials, was the appearance of large

propagated action potentials and tension, but no increase in rate. An increase in rate began several minutes later, and reached a maximum at a time when the amplitude of the action potential was already declining. It thus appears that the factors controlling the rate are quite separate from the factors affecting conduction. The effect of acetylcholine in linking the pacemaker potentials to propagated potentials may well be a quite distinct phenomenon from its effect of slowing the rate at normal temperatures.

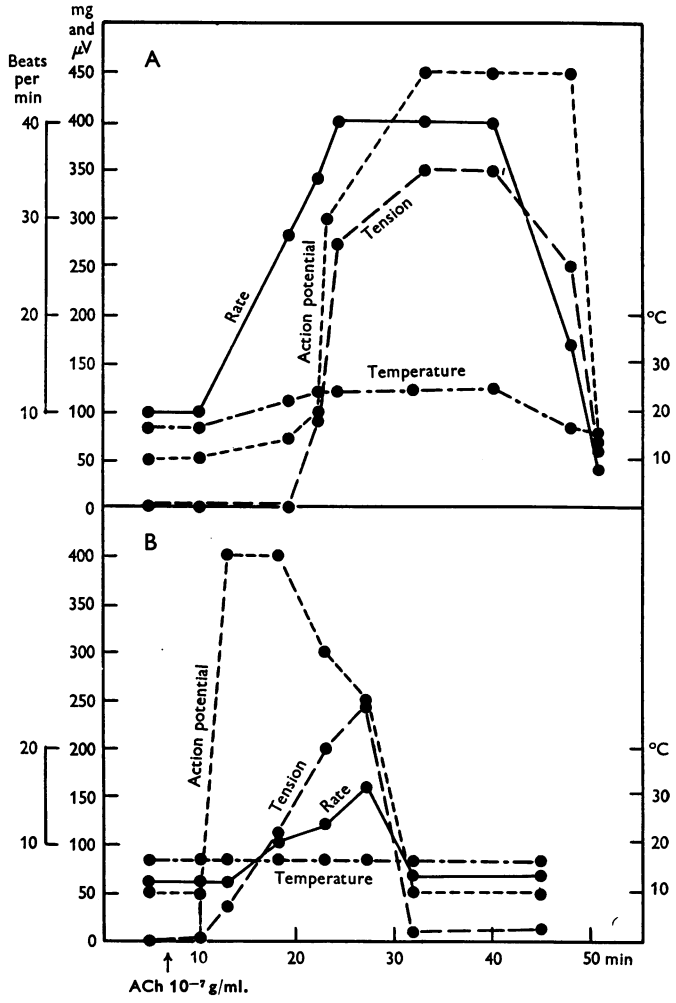

Fig. 7. Rate of beat, height of action potential and tension during change of temperature and application of ACh. A, temperature slowly raised; rate increases gradually but contractions commence at a definite threshold. B, temperature constant at 17° C, ACh 10^{-7} g/ml. added; large action potentials appear suddenly but rate is not affected until auricle has been beating for some time.

DISCUSSION

The mechanism by which the heart beat is initiated has been explained in a number of ways, but direct experimental evidence of relevance has not been easy to obtain. Arvanitaki & Cardot (1937) observed small rhythmical potentials preceding each beat of the snail's heart. Bozler (1943) described pacemaker potentials of two kinds. The first was a low frequency oscillation on which action potentials were superimposed. Acetylcholine was said to slow the heart by increasing the number of oscillations between successive action potentials. Such potentials were only seen in turtle and cat hearts, and were not always found, so that Bozler himself suspected that they might have a traumatic origin (cf. Adrian, 1930). Nothing resembling an oscillation of this kind was seen in our experiments.

The second type of pacemaker potential described by Bozler was a small 'foot' in front of the action potential. This was not an oscillatory potential, but occurred at the normal heart rate, and was found only in the region of the pacemaker. A slow diastolic wave of depolarization preceding the fast upstroke of the action potential was recorded with intracellular capillary electrodes in the sinus node of the frog heart by Trautwein & Zink (1952) and del Castillo & Katz (1955), and similar potentials were observed at the sites of origin of action potentials in spontaneously beating excised Purkinje tissue (dog, goat) by Weidmann (1951, 1955), Trautwein *et al.* (1953) and Coraboeuf & Weidmann (1954). This evidence suggests that the slowly rising foot preceding the action potential would be seen in any region which was firing spontaneously, and that the production of such 'pacemaker' potentials is not the prerogative of a specific anatomical region of the heart.

Rijlant (1932) placed electrodes on the sinus and presinus regions of the intact dog, cat and rabbit hearts, and recorded action currents which preceded the auricular action potentials by 5–20 msec. The interval could be increased by cooling the heart. Our own experiments have revealed at low temperatures in isolated rabbit auricles potentials which seem to have more in common with these sinus potentials in intact hearts than with the 'pacemaker' potentials observed in excised Purkinje tissue. The two types of potential suggest two ways of depicting the auricular pacemaker. First as a specialized tissue, perhaps a remnant of the sinus venosus, whose main function is not to contract, but to produce regularly occurring potentials to which the rest of the auricle can respond with propagated action potentials and contractions. Secondly, as a part of an auricular syncytium, not remarkably different from the rest, which happens to have the fastest spontaneous rhythm, and which, by coincidence, is also the part most resistant to cooling.

The evidence presented is not decisive, but it favours the former view. Small regularly occurring potentials persisted at the pacemaker to a tempera-

ture several degrees below that at which the rest of the auricle ceased to produce potentials or to contract. The potentials were found in a region already located as the origin of the auricular potential at a higher temperature. The pacemaker potentials exhibited a singular regularity of form and time-course, whether the rest of the auricle fired or not. Although no tension was recorded in the presence of the slow pacemaker potentials, as soon as the faster and larger propagated action potentials began to 'take off' from them on re-warming, the development of tension was at once readily observed. When the low temperature was maintained, the pacemaker potentials could be made to fire propagated action potentials by the addition of acetylcholine. This effect was abolished by atropine and potentiated by eserine, but neither atropine nor eserine had any effect on the shape or frequency of the pacemaker potentials themselves.

Since the pacemaker potentials were only distinguishable entities at low temperatures, we have no evidence whether acetylcholine has any function in linking the pacemaker with the rest of the auricle at higher temperatures. It is of interest to recall the hypothesis of Bülbring & Burn (1949), which was the starting point of the present investigation, that the synthesis and release of acetylcholine plays a part in the maintenance of the normal cardiac rhythm. This hypothesis was based upon observations of the rate of synthesis of acetylcholine and the duration of activity in isolated auricles, and upon much other evidence reviewed by Burn (1950). Recently, with the aid of F. J. Philpot (unpublished experiments), it has been found, in preparations of fresh auricles, that acetylcholine synthesis is greater in the neighbourhood of the pacemaker than elsewhere.

Whatever view may be taken of the nature of the pacemaker, the two principal points established by the present work remain. First that activity persists in the pacemaker region at considerably lower temperatures than in other parts. Secondly, that the functional link, broken by cooling, between the pacemaker and the rest of the auricle, can be fully re-established by extremely small amounts of acetylcholine. In the interval, often of a few minutes' duration, between the addition of the acetylcholine and the commencement of propagated potentials associated with measurable contractions, the shape and frequency of the pacemaker potentials were not themselves consistently changed. These facts, together with the other evidence already presented, suggest that the effect of acetylcholine in re-establishing the link between the pacemaker and the rest of the auricle at low temperatures is a phenomenon quite distinct from its usual effect of reducing the rate of the pacemaker at normal temperatures.

How then is the link between the pacemaker and the rest of the auricle restored by acetylcholine? An answer may be given by some experiments at present in progress whose object is to determine whether acetylcholine

causes a slight increase in membrane potential at low temperatures. It has been established that the rate of entry of sodium is a function of the resting potential (Weidmann, 1955). It is possible that conduction fails because the membrane potential falls so low on cooling that sodium entry is too slow to discharge the membrane around the pacemaker. A small increase in resting membrane potential produced by acetylcholine (Burgen & Terroux, 1953*b*; Hoffman & Suckling, 1953) might conceivably be sufficient to permit the membrane current initiated by the pacemaker potential to reach the threshold required to fire off the surrounding muscle.

SUMMARY

1. Isolated rabbit auricles were allowed to beat in a chamber whose temperature could be fixed at any point between 10 and 35° C.

2. Electrical records were taken from the region of the pacemaker in the right auricle and from the tip of the left auricle. Contractions were recorded by a transducer, RCA 5734. The auricles were then slowly cooled from 30° C.

3. At temperatures (14–20° C) at which all other electrical and mechanical activity had ceased, small rhythmical potentials were observed in the region of the pacemaker in nineteen out of twenty-five experiments.

4. As the tissue was rewarmed the first activity seen consisted of small rhythmical non-propagated potentials in the pacemaker region. At a definite threshold, several degrees higher, faster and larger action potentials appeared to 'take off' from the pacemaker potentials. The large potentials were propagated across the whole auricle, and were associated with the development of contractions.

5. When the auricles were kept at a temperature at which non-propagated pacemaker potentials only were observed, acetylcholine 10^{-6} to 10^{-8} g/ml. caused propagated action potentials and full auricular contractions.

6. This effect of acetylcholine could be potentiated by eserine and abolished by atropine. Atropine and eserine alone had no effect on either the rate or amplitude of the pacemaker potentials. Acetylcholine in these concentrations had no effect on the auricles at 30° C.

7. In six experiments no pacemaker potentials were seen at the low temperatures, but the auricle responded to electrical stimulation. If the temperature was lowered further to a point where the response to the stimulus was infrequent, the addition of acetylcholine promptly restored the electrical and mechanical activity to their normal levels.

8. The conclusion drawn was that the pacemaker region was able to resist cooling to a temperature several degrees below that at which the rest of the auricle ceased to contract. The link between the pacemaker and the surrounding tissue, thus broken by cooling, could be restored by acetylcholine.

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