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THE EFFECTS OF ACETYLCHOLINE IN THE HEART-LUNG PREPARATION INCLUDING THE PRODUCTION OF AURICULAR FIBRILLATION

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In a recent paper a description was given of the effect of adding various inhibitors of cholinesterase to the blood circulating through the Starling heart-lung preparation of the dog (Burn & Walker, 1954). Concentrations of 10^{-6} M to 10^{-5} M of eserine and other inhibitors reduced the rate to 60–70% of the initial value and the reduction was abolished by very small amounts of atropine. Since central control is absent in the heart-lung preparation these results suggested that acetylcholine (ACh) formed locally in the heart was influencing the rate, since it was slowed in the presence of inhibitors of cholinesterase of widely differing structure.

The action of ACh in the heart-lung preparation has now been studied both when the heart was beating spontaneously and when the rate was controlled by electrical stimulation of the auricle; auricular and ventricular rates were measured from electrocardiograms. Observations have been made on the effect of ACh on the heart rate and output and on the production of block. In the course of the work we found that we were able to produce and to stop auricular fibrillation at will.

Fibrillation has been produced by many methods. Rothberger & Winterberg (1910) produced it by rapid stimulation of the auricles in the heart usually treated with nicotine, but sometimes untreated. Lewis, Feil & Stroud (1920) induced flutter by rhythmic stimulation of the auricles rising to a critical rate between 350 and 500 per min. de Boer (1921) produced ventricular fibrillation in the heart of the frog by applying a single induction shock at the beginning of the excitable period after a previous contraction. Andrus & Carter (1930) produced auricular fibrillation in the dog's heart by a single stimulus introduced shortly after the end of the refractory period during vagal stimulation. Brams & Katz (1931) induced auricular flutter and fibrillation by

short faradic stimulation applied to the right auricle of the dog. Wiggers & Wegria (1940) produced ventricular fibrillation in the dog by a single induction shock applied during the 'vulnerable' phase of ventricular systole, which was again shortly after the end of the refractory period. Nahum & Hoff (1940) produced auricular fibrillation by application of acetyl-β-methylcholine chloride to localized regions on the auricular surface. Rosenblueth & Garcia Ramos (1947) produced auricular flutter by brief rapid electrical stimulation near a crushed area on the right auricle. Scherf, Romano & Terranova (1948) produced flutter and fibrillation by the injection of a dilute solution of aconitine into the dog's auricle or by the application of crystals of aconitine to the surface. Scherf & Chick (1951) showed that when a 5% solution of acetylcholine was applied to the area of the sinus node flutter or fibrillation started immediately. Dawes & Vane (1951) produced repetitive discharges in the isolated auricles of guinea-pigs by stimulating the preparation at a rate slightly greater than the normal rate, and throwing in another stimulus after every fourth beat. When this fell just outside the absolute refractory period the auricle responded by repeated discharges at a much greater rate than the normal.

It is clear, therefore, that flutter or fibrillation can be precipitated in a number of ways. In many of the experiments mentioned above the phenomenon was not regularly observed, nor, when the condition was induced, could its cessation be controlled. Perhaps the main points of interest concerning the technique described in this paper are that fibrillation could always be induced, and could subsequently be turned off or on at will.

METHODS

The preparation was made by the method described by Knowlton & Starling (1912). Two dogs were used for each experiment. The first was anaesthetized with ether and its blood was collected from the carotid artery. The second was also anaesthetized with ether, and some blood was withdrawn and added to the blood of the first, to form a reservoir for the experiment. The preparation of the heart-lung in the second dog then proceeded under chloralose (0·07 g/kg), but, since the reservoir blood used during the experiment had been exposed only to ether (which was soon removed in the expired air), the final concentration of anaesthetic consisted of probably less than 70 mg chloralose/l. blood. The venous reservoir, which was immersed in a constant temperature bath, contained an overflow tube above which the level of blood could not rise. The overflow tube led the excess blood to a second reservoir placed below the first; it was then returned to the top reservoir by a Dale-Schuster (1928) pump. Thus a constant venous pressure was ensured. The blood was prevented from clotting with heparin (Boots) 5000 units/l. The temperature in the venous cannula was recorded at 5 min intervals throughout each experiment, and the heating of the bath was adjusted to keep it constant.

Electrocardiograms (e.c.g.) were taken on a Cossor machine, model 1314, which employs a Kelvin-Hughes pen writing directly on Teledeltos paper. The leads were attached to the right and left forelegs and the left hindleg after removal of some skin. The auricles were made to beat at any required frequency by means of a square-wave stimulator of fairly conventional design. Constant current pulses of duration 0·9 msec and strength 1 mA were employed. The stimulator had two independent channels, each variable with respect to frequency, delay, duration and strength. It

was thus possible to send a second pulse, synchronized with the stimulus driving the auricle, to a separate pen placed at the edge of the electrocardiograph paper.

The electrodes first used were a pair of fine fish-hooks which pierced the auricular tissue. They were later replaced by electrodes fixed on each side of the upper jaw of a spring clip which gripped the tip of the right auricle. The electrodes were made of rounded platinum wire almost flush with the insulating material in which they were embedded. The spring of the clip was sufficiently strong to prevent any movement of the electrodes due to the contractions of the auricle.

Artifacts due to the stimulus were prevented from reaching the e.c.g. trace by the employment of a specially wound isolation transformer designed to give low capacitance to ground in spite of being able to handle fairly large currents. A certain amount of ring was encountered originally, but was reduced to unimportant levels by means of a suitable shunt resistance. To apply the electrodes to the right auricle, a triangular piece of pericardium of side about 2 cm was removed. The pericardium was not disturbed over the rest of the heart. For infusion of solutions of ACh into the blood, we used an apparatus similar to that described by Burn & Dale (1924). A burette was almost filled with the solution. Above it liquid paraffin entered the burette through a tube in the rubber stopper closing the burette. The liquid paraffin came from a Marriotte bottle in which it was under a known air pressure. Grooves in the ground glass of the burette tap provided a capillary of variable length which made it possible to infuse at constant rates from 0·2–2·0 ml./min. The solution entered the blood through a syringe needle piercing the rubber tube attached to the cannula in the superior vena cava.

RESULTS

Effect of ACh infusion on rate. To compare the effect of ACh on the natural rate of the heart with the effect of the inhibitors of cholinesterase described previously we infused a solution of ACh at a constant rate into the blood entering the cannula in the superior vena cava. Observations were made in eight preparations, and the results are given in Table 1. For example, five observations were made in which ACh was infused at the rate of 400 μg/min. The heart rate was unchanged in two of the observations and slightly reduced in three; the heart rate during infusion is shown in Table 1 as a percentage of the rate before infusion. The mean value of the five observations was 95%. Table 1 shows that the infusion of ACh had little effect on the heart rate in amounts up to 400 μg/min. In one experiment the very large amount of 1·6 mg/min was infused. This reduced the heart rate from 146 to 114/min, that is, to 77% of the initial value. In the earlier paper (Burn & Walker, 1954) it was shown that the addition of eserine 3×10^{-6} M or 1 mg/l., reduced the rate to a mean of 67% of the initial value in seven experiments.

In two experiments we tested the effect of ACh on the heart rate both before and after the addition of hexamethonium to the blood. In the first experiment 5 mg was injected and in the second 10 mg was injected, the total volume of blood being 1 l. The slowing produced by ACh infused at increasing rates was not appreciably modified by the hexamethonium.

Effect of ACh on the output. We measured the output directly by collecting the blood in a measuring cylinder for periods of 15 sec. To determine the effect of ACh on output we kept the heart rate constant by stimulation, and tested the effect of infusing ACh both before and after adding hexamethonium.

Observations taken from seven experiments are shown in Table 2 and when the figures in the last column are compared with those in the last column of Table 1 it is clear that ACh diminished output to a greater extent than it diminished the spontaneous rate. In any one experiment the fall in output was proportional to the rate of infusion. In different experiments, however, the effect on output varied greatly for the same rate of infusion, and in consequence the figures for the mean percentage were not graded according to the amount

TABLE 1. Effect of ACh infusion on heart rate

Amount infused (μg/min)	Heart rate during infusion as percentage of initial rate. Figures from different experiments	Mean percentage
50	92	92
100	100, 100, 94, 97, 100	98
200	96, 100, 85, 85, 92	92
400	100, 95, 95, 100, 84	95
800	85, 92, 86	88
1600	77	77

TABLE 2. Effect of ACh infusion on systemic output, determinations made at constant rate

Amount infused (μg/min)	Output during perfusion as percentage of initial output. Figures from different experiments	Mean percentage
60	68, 83	75
94–133	79, 86, 33, 80, 70	69
184–236	80, 41, 64	62
364–400	66, 87, 100	84
800	81, 46	63

infused. However, the mean of the figures in the last column of Table 1 is 90, while the mean of the figures in the last column of Table 2 is 70. When hexamethonium was present in amounts up to 10 mg/l., the effect of ACh on output was not modified.

Auriculo-ventricular (A-V) block produced by ACh. In six out of fourteen experiments in which infusions of ACh were made, the infusion produced a fluctuating A-V block. This is illustrated in Fig. 1 which shows the record of the pressure in the brachio-cephalic artery obtained after the addition of 10 mg hexamethonium to the blood in the reservoir. This addition was without effect. In Fig. 1*a* ACh was infused at the rate of 52 μg/min. The intermittent fall in the blood pressure was due to a transitory 2 : 1 block which reduced the ventricular rate to half. At the beginning of the infusion the heart rate was 153; it fell to 135 and then to 128, and at this point the block began; for the duration of 4 beats the ventricular rate fell to 64; for the next 25 beats the block was absent and the ventricular rate rose to 128; then again for 5 beats the ventricular rate fell again to 64, and so on. In Fig. 1*b* the duration of the 2 : 1 block was longer, and in Fig. 1*c* it was still longer. Thus the following

sequence was observed on the electrocardiogram; V (ventricular rate) = A (auricular rate) for 8 beats; V = ½A for 21 beats; V = A for 12 beats; V = ½A for 21 beats; V = A for 10 beats. Finally, in Fig. 1*d*, when the rate of infusion was 240 μg/min there was not only a 2 : 1 block for most of the time, but a 4 : 1 block also began to make its appearance. The change from 1 : 1 to 2 : 1, then to 4 : 1 and back to 1 : 1 nevertheless continued. The block was always associated with a prolongation of the *P-R* interval.

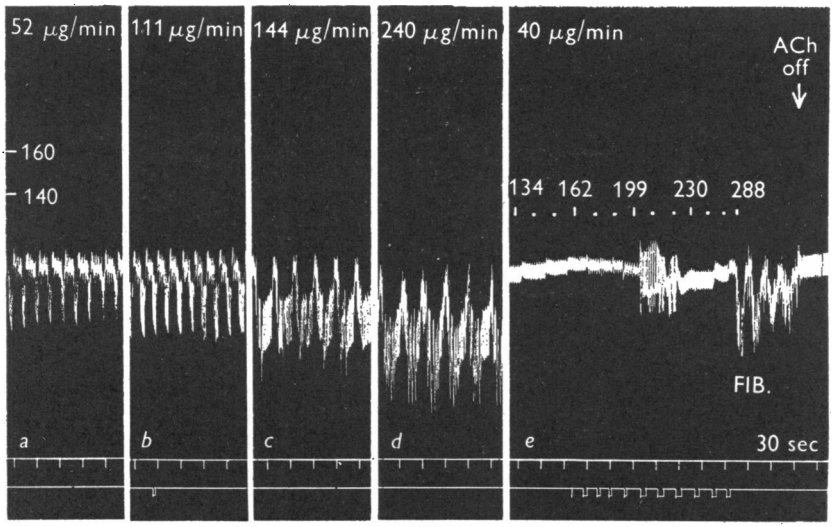

Fig. 1. Dog heart-lung preparation. Record of pressure in the brachio-cephalic artery during the infusion of ACh into the superior vena cava. The effects were all observed after the addition of 10 mg hexamethonium to the blood in the reservoir. (*a*) Infusion of 52 μg/min caused fluctuating block when the ventricular rate fell to half for short periods; (*b*) infusion of 111 μg/min led to block of longer duration; (*c*) infusion of 144 μg/min and (*d*) infusion of 240 μg/min introduced a transitory 4 : 1 block; (*e*) infusion of 40 μg/min which did not cause block. Stimulation applied at increasing rates from 122/min upwards. At the rate of 288/min, the e.c.g. showed that the auricles were fibrillating; fluctuations in the ventricular rate still occurred and caused the changes in blood pressure. Fibrillation stopped when the infusion of ACh was stopped.

In experiments in which the infusion of ACh did not produce a block when the heart rate was determined by the pacemaker, a block was produced when the heart was driven electrically. This is illustrated in Fig. 2 which is an e.c.g. taken during the infusion of ACh at the rate of 13 μg/min and when stimulation was applied at 150/min. In the first half of Fig. 2 there was a 2 : 1 block, which then gave way to a 1 : 1 rhythm for six beats, and then the 2 : 1 block returned.

The depression of A-V conduction by ACh could be shown in another way. Before the infusion of ACh was begun, the auricle was stimulated at increasing rates. Starting at a point just faster than the spontaneous rate, the rate was

increased in steps of approximately 10%, the auricle being allowed to beat for about 40 sec at each rate. At the lower rates the ventricle followed the auricle, so that a ratio of 1 : 1 was maintained. But at a certain point (255/min in the experiment of Fig. 3), which varied greatly in different dogs, but which remained fairly constant in successive runs on the same dog, the ventricular rate

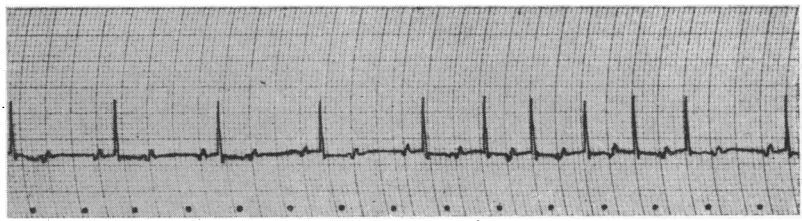

Fig. 2. E.c.g. during infusion of ACh at rate of 13 μg/min, showing fluctuating block caused by stimulation at 150/min. The time of arrival of the stimulus corresponds to the point at which a line drawn at right angles to the edge of the paper through the stimulus mark cuts the centre line of the e.c.g. trace. E.c.g. from leads on right foreleg and left hindleg, in this and later figures.

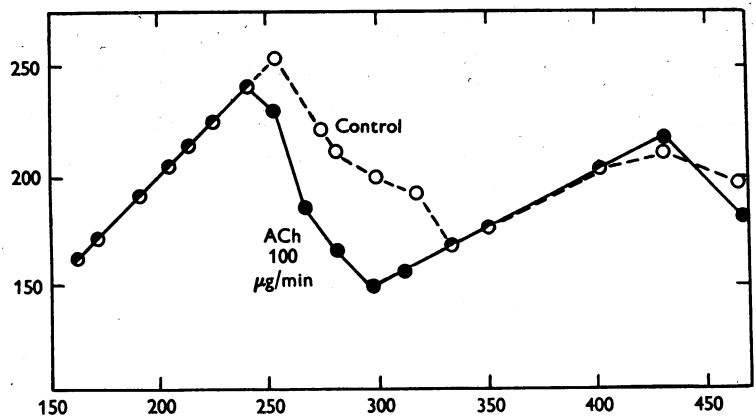

Fig. 3. Effect of ACh in producing A-V block. Abscissa: rate of stimulation of auricles per min. Ordinate: ventricular rate per min. In control observations the ventricles followed the auricles up to 255/min. As the driving rate rose further the ventricular rate fell until a 2 : 1 rhythm was seen at 330/min. The 2 : 1 rhythm was maintained up to 400/min etc. See text.

fell, while the auricular continued to rise. That is to say, at regular intervals which became shorter the higher the rate of stimulation, an auricular beat was not conducted to the ventricles. For any one rate of stimulation the intervals were constant, so that the percentage of auricular beats which were followed by ventricular beats did not fluctuate as in the block described in the preceding paragraph. Fig. 3 shows that when stimulation was 330/min the ventricular

rate fell to half the auricular rate and remained at this proportion up to 400/min, above which it again fell to a slightly lower proportion.

When the stimulation was repeated during the infusion of a solution of ACh the maximum rate at which the ventricles followed the auricles was lower, and the fall in ventricular rate took place at lower rates of stimulation. The rate at which a 2 : 1 block became established was 300/min instead of 330/min.

A large number of observations on the effect of ACh infusions was made in this way and these have been summarized in Table 3. The effect of ACh was extremely variable from one experiment to another. For example, in three experiments an infusion was made at a rate between 184 and 200 μg/min. In one of these the rate of stimulation above which the ventricle failed to follow every contraction of the auricle fell by 50 beats/min (from 272 to 222 beats/min) in the presence of ACh. In another the rate did not fall at all in the presence of ACh. The same variation from one experiment to another was seen when the rate of infusion was from 364 to 400 μg/min.

TABLE 3. Effect of ACh infusion on A-V conduction

Rate of infusion (μg/min)	Fall in maximum rate of stimulation at which V = A (beats per min)	Mean fall	Fall in minimum rate of stimulation at which V = ½A (beats per min)	Mean fall
40–60	30, 0	15	46, 8	27
94–100	16, 0, 4, 9, 2	6	46, 0, 38, 24, 0	22
184–200	14, 50, 0	21	4, 88, 28	40
364–400	15, 117, 0, 22	38	61, 98, 0, 42	50
800	45	45	72	72
1728	142	142	138	138

Auricular fibrillation. In the earlier experiments in which fish-hook electrodes were used for stimulation, rates of stimulation up to 512/min and 680/min caused auricular fibrillation in two experiments respectively. It seemed possible that the injury of the auricle was responsible for the fibrillation, and the spring-clip electrodes already described were used thereafter. With these electrodes stimulation at rates up to 600/min did not cause auricular fibrillation in any experiment. When the electrodes were removed from the auricle after a period of 2 hr only a minor bruising indicated the site of attachment.

We found, however, that even with these electrodes, producing no apparent injury, auricular fibrillation could be produced at will, if the auricles were stimulated at a fast rate in the presence of an appropriate concentration of acetylcholine. Once the fibrillation was established, turning off the stimulus had no effect, and the fibrillation continued until the infusion of ACh was stopped or greatly reduced. In Fig. 1*e* ACh was infused at a rate insufficient to cause the fluctuating block shown in the earlier sections of Fig. 1, and stimulation began at the rate of 122/min. The rate was increased without stopping stimulation by steps of about 10 beats/min, up to 134, which is the first rate marked in the figure, and then further as shown by each spot until

the rate of 288/min was reached. 199/min was the rate above which the ventricle ceased to follow all beats, and 230/min was the rate at which a 2 : 1 rhythm began. At 288/min the auricles began to fibrillate, and the fibrillation continued when the stimulation was stopped. After $1\frac{1}{2}$ min the infusion of ACh was stopped and after 38 ventricular beats the normal rhythm returned.

In another experiment stimulation at rates up to 384/min did not cause fibrillation during the infusion of ACh at 96, 184 and 364 μg/min. However, fibrillation occurred during the infusion of 800 μg/min when the stimulation reached the rate of 222/min. It continued when the stimulation stopped, but when the infusion stopped the normal rhythm returned. Fig. 4 shows what

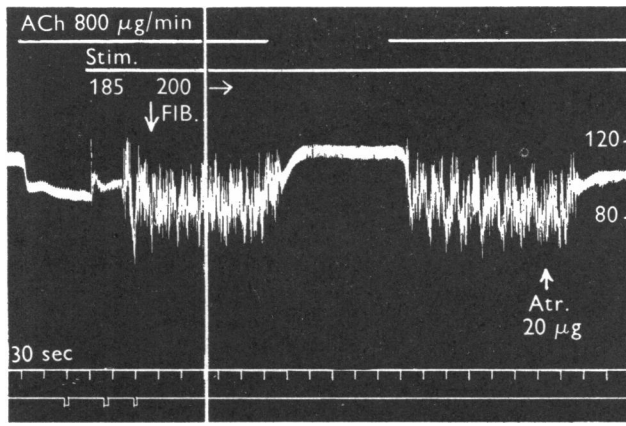

Fig. 4. Blood pressure record of heart-lung preparation. Auricular fibrillation occurred during the infusion of ACh at 800 μg/min when stimulation was applied at the rate of 200/min. When the infusion of ACh was stopped the fibrillation stopped although the stimulation continued. When the infusion started again, the fibrillation began again; it was arrested by the injection of 20 μg atropine.

happened after this. The infusion was restarted and fibrillation began again when the stimulation rate was 200/min. Arrest of the infusion of ACh stopped the fibrillation, although the stimulation continued, and then the restart of the infusion for a third time brought back the fibrillation. The injection of 20 μg atropine then stopped the fibrillation although the infusion of ACh and the stimulation both continued.

Regular production of fibrillation. By stimulating the auricles during the infusion of ACh we have produced auricular fibrillation in eleven experiments and we have had no failures. Once the fibrillation began it continued so long as the infusion of ACh continued, even for as long as 1·5 hr. When the infusion was stopped the auricles always reverted to a normal rhythm. The only limit to the length of time for which the fibrillation was maintained was the condi-

tion of the preparation; when lung oedema occurred the experiment naturally could not continue. In one preparation used in a demonstration to the Physiological Society, fibrillation was produced and stopped 6 times.

The procedure can be followed from Figs. 5–7. In Fig. 5, part 1, before infusion of ACh and without electrical stimulation the rhythm was regular at 165/min. In Fig. 5, part 2, infusion of ACh at 400 μg/min produced block; five auricular waves were not followed by ventricular complexes, and one auricular

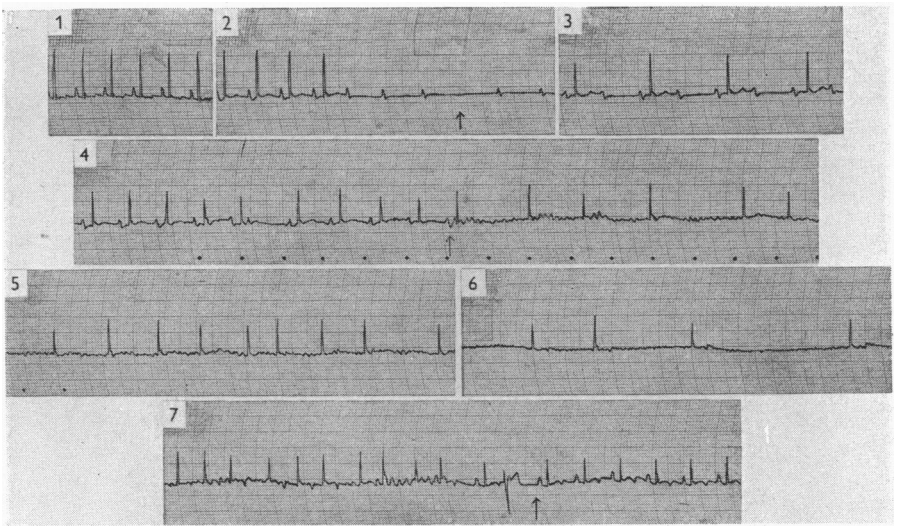

Fig. 5. E.c.g. (1) Normal rhythm. (2) Block produced by infusing ACh at 400 μg/min. An auricular beat was missed at the arrow. (3) Block during infusion of 200 μg/min. (4) Infusion of ACh 100 μg/min without block. Dots denote stimulation (117/min). Fibrillation began after 7th stimulus. (5) and (6) Fibrillation continued though stimulation was stopped. (7) Reversion to normal rhythm at arrow after infusion was stopped. Note flutter before reversion to normal rhythm. Leads right foreleg, left hindleg.

wave was missing (at the arrow). In part 3 the infusion of ACh was reduced to 200 μg/min and the block became 2 : 1. In part 4 the infusion of ACh was reduced further to 100 μg/min, so that block was absent, the heart rate being 128/min. Stimulation then began at the slower rate of 117/min, and fibrillation was seen after the 7th stimulus. In part 5 the stimulation was stopped but the fibrillation continued. In part 6 the fibrillation continued with a slower ventricular rate. The infusion was stopped and in part 7 the fibrillation was converted to the normal rhythm. The first normal contraction was followed by an extra-systole and then at the arrow normal rhythm began.

In another preparation which was more sensitive, the infusion of 8·5 μg ACh/min caused a fluctuating block when stimuli were applied at 376/min. When stimulation was 562/min the block was 4 : 1. In spite of the block, the

rate of ACh infusion was raised to 11 μg/min and stimulation at rates up to 563/min was tried again. The ACh infusion was then increased to 22 μg/min, to 35 μg/min, to 55 μg/min, to 85 μg/min and finally to 165 μg/min, stimulation being applied at rates up to 562/min at each of the rates of infusion.

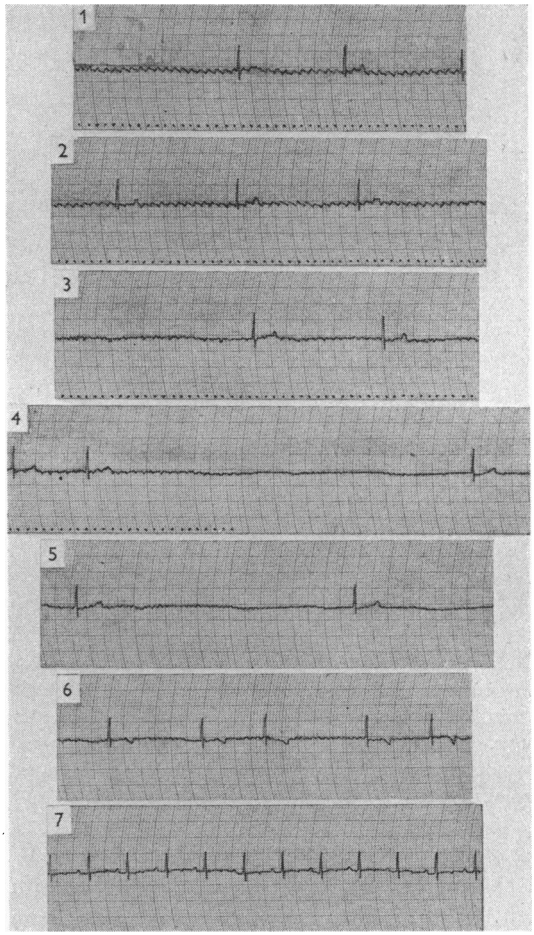

Fig. 6. E.c.g. Stimulation indicated by dots at the rate of 562/min. In (1) ACh was infused at 55 μg/min. In (2) the rate of infusion was 85 μg/min and in (3) 165 μg/min. In (1) and (2) the auricles followed the applied stimuli. In (3) the auricles partly followed, but fibrillation was visible in places. In (4) when the stimulation stopped the auricles were fibrillating. In (5) fibrillation continued. ACh was then turned off. In (6) the ventricular rate increased and in (7) there was a normal rhythm.

Parts 1, 2 and 3 of Fig. 6 show the e.c.g.'s taken during infusion at 55, 85 and 165 μg/min respectively, when stimulation was applied at 562/min. In part 1, the auricles clearly followed each stimulus; in part 2 the auricles still followed,

but the auricular spikes were less well marked, and in part 3 the auricular spikes were sometimes following, but there was a background of fibrillation as well. In part 4 the mixture of obedience to the applied stimulus and of fibrillation was made evident when the stimulation was stopped, for only fibrillation remained. In part 5 the fibrillation continued. The infusion of ACh was then stopped with the resulting increase in ventricular rate seen in part 6 and the return to normal rhythm seen in part 7. In this experiment fibrillation was produced again and it continued for 45 min, during the infusion of ACh.

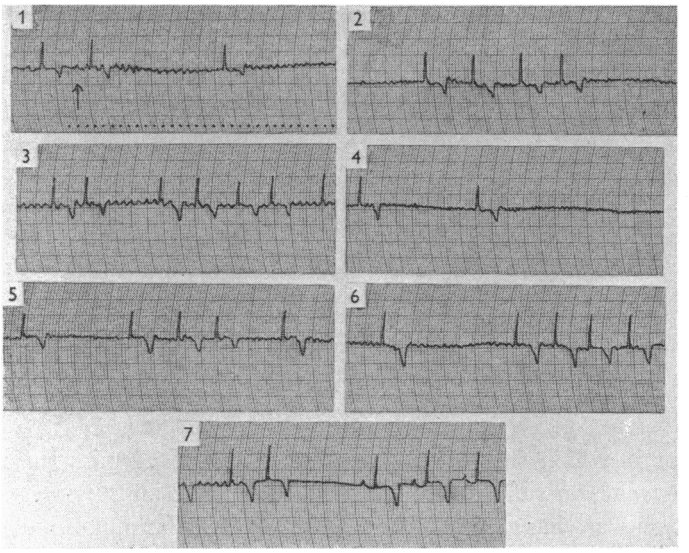

Fig. 7. E.c.g. Infusion of ACh at 2·4 mg/min. In (1) the last normal *PRT* is shown before stimulation began at 570/min. Fibrillation occurred after the 2nd stimulus, continuing in (2) when stimulation was stopped. In (3) the rate of infusion of ACh fell, the fibrillation turning to flutter. In (4) the rate of infusion was restored to 1·5 mg/min and fibrillation was seen again. (5) was 30 min after (4). (6) was 53 min after (5). Return of normal rhythm after 1·5 hr in (7) when ACh was turned off.

The records in Fig. 7 have been included for three reasons. As shown in part 1 fibrillation occurred after the application of the second stimulus; indeed possibly after the first. Between parts 2 and 3 the rate of ACh infusion was reduced in changing from one infusion apparatus to another, with the result that the fibrillation seen in part 2 changed to the flutter seen in part 3; on raising the infusion rate to 1500 μg/min fibrillation returned as seen in part 4. Finally fibrillation was maintained by ACh infusion for 1½ hr, part 5 being 30 min after part 4, and part 6 being 53 min after part 5. The infusion of ACh was then stopped and normal rhythm returned as shown in part 7. Fibrillation was started again twice more by turning on the infusion and stimulating the

auricle. In this experiment an attempt was made to induce fibrillation by throwing in a single stimulus, since in two experiments we observed that the second of a series of stimuli started fibrillation. The single stimulus was unsuccessful perhaps because it did not arrive at the right moment; a series of stimuli were, however, effective.

The figures for the amounts of ACh infused per min and the rates of stimulation per min necessary to cause fibrillation in the different experiments are given in Table 4. The amounts of ACh were those infused at the onset of fibrillation. In those experiments in which fibrillation was maintained for some time we found that the rate of ACh infusion could be reduced as the experiment continued without a reversion to normal rhythm. For example, in the

TABLE 4. Production of auricular fibrillation

Amount of ACh infused (μg/min)	Rate of stimulation per min
40	288
112	117
165	562
200	98
200	266
800	100
800	222
800	384
1600	214
2400	570
3200	85

experiment in which the rate at the onset was 165 μg/min, the infusion of 17 μg/min was later sufficient to maintain the fibrillation. By watching to see if fibrillation was changing to flutter and if ventricular spikes were becoming very frequent it was possible to know whether the rate of infusion of ACh had been reduced too far, and if so to increase it once more, and so to re-establish the fibrillation.

DISCUSSION

For the study of factors affecting the heart beat, the heart-lung preparation offers several advantages. It works without an anaesthetic, it is free from central nervous control, it is free from hormonal influences, and it is free from an external vascular system other than the pulmonary vessels. Thus it is possible to test the action of acetylcholine in amounts which could not be given to the whole animal. The results showed that even large amounts of acetylcholine had very little effect on the heart rate, but had more effect in reducing the systemic output.

Acetylcholine also depressed A-V conduction as was shown in two ways. First it was observed that it produced a fluctuating block in six experiments out of fourteen when the heart rate was controlled by the pacemaker. The rates of infusion which had this effect varied from 13 μg/min to 3·2 mg/min.

The fluctuations were difficult to understand since the rate of infusion was constant. It is, however, possible that the fluctuations were due to changes in the rate at which the blood passed through the heart. When block occurred, the ventricular beats became less frequent, so that the transfer of blood from the venous cannula to the right auricle must have been slower. Hence there would be more time for the cholinesterase in the blood to reduce the concentration of ACh present, and therefore the block might pass off, to return again as the ventricular rate quickened.

Secondly, the effect of acetylcholine on A-V conduction was evident when the heart was driven electrically at an increasing rate. At the lower rates, the ventricles followed the auricles; above a certain rate the ventricular rate gradually fell as the auricular rate rose until the ventricular rate was half the auricular rate. When the observations were repeated during an infusion of acetylcholine at a rate insufficient to produce the fluctuating block already described, the rate of stimulation at which the ventricles ceased to follow the auricles was lower; a 2 : 1 block was also established at a lower rate. But it was noteworthy that the effect of acetylcholine was extremely variable. Rates of infusion as high as 800 μg/min while having a large effect in one experiment had almost none in the next. One explanation that suggests itself is that the amount of circulating cholinesterase varied greatly in different dogs.

When the auricles were stimulated electrically it was always possible to find an amount of acetylcholine and a rate of stimulation which together produced auricular fibrillation. Once fibrillation began it continued, although the stimulation was stopped, and persisted so long as the acetylcholine was infused. As has been stated, in our early experiments we used fish-hook electrodes, and we produced auricular fibrillation with them on two occasions by stimulation alone. When electrodes which were carried on a spring clip were substituted for the fish hooks, we did not observe fibrillation in any experiment as a result of stimulation up to 600/min. We, therefore, suspected that when electrodes were used which caused trauma, as the fish-hook electrodes did, fibrillation was more liable to develop as a result of the trauma. When we used electrodes which caused less trauma, auricular fibrillation occurred in response to stimulation during the infusion of acetylcholine only. Fibrillation then often followed the application of a few stimuli.

Since in the experiments of de Boer (1921), Andrus & Carter (1930) and of Wiggers & Wegria (1940) only one stimulus was needed to cause fibrillation provided it was applied at the right moment, it is probable that a few stimuli were necessary in our experiments in order that one of them should be suitably timed. If this is so we must then seek to answer the question why a single stimulus applied to an auricle exposed to the action of acetylcholine should cause fibrillation. To attempt to answer it we must consider what is known of the part played by acetylcholine. In 1887 Gaskell showed that when he

recorded the demarcation current of the injured auricle of the tortoise heart, stimulation of the vagus nerve caused a large increase in the current. Burgen & Terroux (1953) recently found that acetylcholine produced a state of hyperpolarization in strips of cat's auricular muscle, thus supporting Gaskell's result. The question arises whether there is any evidence that hyperpolarization facilitates the appearance of fibrillation. Of obvious relevance is the work of Harris & Moe (1942) who showed that anodal polarization of the dog's ventricle by a constant current led to discrete ectopic beats which were followed by fibrillation.

The factors concerned in the production of fibrillation may be a shortening of the refractory period by the acetylcholine, and also the appearance of a supernormal phase of excitability, first demonstrated in cardiac tissue by Adrian (1920). The importance of the supernormal phase was emphasized by Segers (1947), who studied the effect of applying electrical stimuli to the isolated left auricle of the rabbit which does not beat spontaneously. He showed that under the influence of adrenaline or of calcium a series of stimuli applied and then stopped would fire off a succession of spontaneous contractions and that the appearance of these contractions was related to the summation of successive supernormal phases. Since, however, the contractions observed involved the auricle as a whole, their relevance to the condition of auricular fibrillation is uncertain.

The onset of fibrillation was not a sudden and complete event in all of our experiments. It was sometimes observed, for example, that with a fixed rate of stimulation the auricular waves diminished in size as the concentration of acetylcholine was increased, implying that fewer fibres were producing potentials in phase. With lower concentrations a full-sized auricular wave occurred in response to each stimulus; then with higher concentrations a fibrillation began to appear superimposed on the wave and the wave became smaller. The picture seemed to be explained by the view that fibrillation had begun in part of the auricles while the other part of the auricles continued to follow the stimuli. When the stimulation was stopped, fibrillation only was seen. Reintroduction of the stimulus had no effect since those fibres which had been in phase with the stimulus were now also fibrillating.

Rothberger & Winterberg (1915), and others since, have shown that vagal stimulation converts flutter into fibrillation. The converse of this was repeatedly seen in our experiments. When fibrillation had been established, and the stimulus withdrawn, if the ACh infusion was stopped or slowed, the fibrillation always passed into flutter before a normal rhythm was re-established. Full-sized auricular waves were seen, occurring at a fast rate and followed at varying intervals by ventricular complexes.

The consequences of the presence of acetylcholine in the immediate environment of cardiac muscle are many. The resting potential is elevated; the

refractory period is reduced; A-V conduction becomes depressed and block supervenes. In spontaneously beating isolated rabbit auricles, where A-V conduction is not involved, the presence of acetylcholine causes an increase in conduction velocity (Vaughan Williams, 1954). Measurements of potentials recorded from the inside of cat auricle fibres have shown that acetylcholine increases the rate of repolarization of the membrane after the 'spike' of the action potential (Burgen & Terroux, 1953).

In spite of the detailed knowledge acquired concerning changes in refractory period, in conduction velocity etc. produced by acetylcholine, their relevance to changes in function is unknown. For the fact remains that, so far, only two functional changes produced by acetylcholine have been described, namely reductions in the rate and in the force of the beat. Our experiments in the heart-lung preparation, however, seem to have revealed a third functional effect of acetylcholine, such that if a stimulus arrives in the presence of an appropriate concentration, fibrillation is precipitated. If this can be regarded as an increased excitability it is likely to be related in some way to the decrease in the refractory period and to the faster rate of repolarization of the membrane, but it is impossible to describe how at the present time. The production of an increased excitability is clearly a very different function from the reduction in rate and force of beat, and is in itself sufficient to show that the simple view that acetylcholine is merely inhibitory to the heart is incorrect. In the act of increasing excitability by some combination of the changes described, acetylcholine must be stimulating or enhancing certain phases of the cardiac mechanism.

When we consider the heart as a functioning organ, the greater readiness of the auricle to respond to a stimulus in the presence of acetylcholine is clear. It is then a fact of prime importance that this effect, implying a decrease in the stability of the membrane, is produced by a substance which also slows the pacemaker. The effect of acetylcholine on the rate may thus be regarded as protecting the heart muscle from the dangers inherent in the production of a less stable membrane. If, however, the slowing effect on the rate is nullified artificially by driving the heart at a fast rate with electrical stimuli, then the danger is revealed and the membrane is thrown into an oscillatory condition; either as flutter, when the auricular muscle oscillates as a whole; or as fibrillation, when small groups of fibres oscillate individually.

SUMMARY

1. The effects of infusing acetylcholine into the blood entering the heart-lung preparation of the dog have been investigated when the heart was beating normally and when it was driven electrically. An e.c.g. was taken and the outflow was measured.

2. The infusion of even large amounts of acetylcholine diminished the spontaneous rate only slightly, but it reduced the systemic cardiac output.

3. The infusion of acetylcholine depressed A-V conduction; the degree of depression varied greatly in different experiments.

4. The principal finding was that, during the infusion of acetylcholine, stimulation of the right auricle caused the onset of auricular fibrillation which continued when the stimulation stopped. The fibrillation persisted so long as acetylcholine was infused, and it was maintained for periods up to 1·5 hr. In all experiments fibrillation reverted to normal rhythm when the infusion was discontinued. The fibrillation was stopped by atropine. Fibrillation was produced in all of eleven experiments. In the course of a single experiment fibrillation could be started and stopped several times.

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