Br. J. Pharmac. Chemother. (1967), **31**, 523-536.

EFFECT OF ADRENALINE ON THE VENTRICULAR FIBRILLATION THRESHOLD IN THE ISOLATED RABBIT'S HEART

BY

M. MacCONAILL AND M. F. MURNAGHAN

From the Department of Pharmacology, University of Ottawa, Ottawa, Canada, and Department of Physiology, University College, Dublin, Ireland

(*Received July* 27, 1967)

Since Levy (1911) showed that adrenaline injections could precipitate ventricular fibrillation in cats under light chloroform anaesthesia, much work has been done on the liability of this substance to produce ventricular arrhythmias and fibrillation. On the one hand, Riker, Depierre, Roberts, Roy & Reilly (1955) demonstrated that a sensitizer such as cyclopropane would permit adrenaline to precipitate fibrillation in otherwise ineffective doses; on the other, Hoffman, Siebens, Cranefield & Brooks (1955) found a temporary fall in the electrical ventricular fibrillation threshold *in vivo* during adrenaline infusions in dogs anaesthetized with pentobarbitone. This finding contrasts with that of Wégria & Nickerson (1942), who had earlier found that adrenaline produced increases in the fibrillation threshold *in vivo*. Goodford (1958) had shown that adrenaline aided the production of ventricular fibrillation in isolated rabbit hearts under a particular stimulus pattern. The rise in blood pressure produced by the administration of adrenaline had also been implicated in the production of the arrhythmias, and, recently, Dresel (1962) has shown that atrioventricular blocks arising from vagal action could increase the propensity of adrenaline to produce arrhythmias in dogs.

We therefore decided to measure the effects of adrenaline infusions, alone and in the presence of chloroform and a parasympathomimetic drug such as carbachol, on the facility with which ventricular fibrillation could be induced. The isolated perfused heart of the rabbit was chosen as a suitable preparation for this investigation; the complicating effects of the homeostatic reflex influences present in the whole animal were thus avoided. Preliminary tests showed that apparently simpler methods (Armitage, Burn & Gunning, 1957a; Szekeres & Lénárd, 1960) of measuring the susceptibility of the ventricles to the induction of fibrillation were not in fact as useful as the single electrical pulse fibrillation threshold method of van Tyn & MacLean (1961). This last mentioned method was therefore adapted to the isolated heart, and the significance of the thresholds so measured checked by examining their variation as a consequence of the alteration of the potassium concentration of the perfusing fluid before proceeding to the measurement of the effects of adrenaline.

Preliminary communications of parts of this work were presented to the Canadian

Federation of Biological Societies at Halifax, Nova Scotia, in 1964, and to the Physiological Society of Great Britain and Ireland at Galway, Ireland, in 1965.

METHODS

Perfusion

Isolated rabbit hearts were perfused with McEwen solution (1956) and variants thereof at 37° C and a pressure head of 54 cm. The apparatus was arranged so that the solution perfusing the heart could be selected from three compositions available by a tap: changing took place with the minimum of undesired disturbance in temperature and pressure, and the new solution reached the heart within a few seconds. The path of the perfusion fluid was contained within the controlled temperature system until its entry into the short nylon aortic cannula. A fine polyethylene tube was passed through this cannula so that drugs or solutions could be infused directly into the aorta of the perfused heart. In those experiments where several concentrations of potassium were to be used a common potassium-free stock was first prepared, and then the required amount of potassium chloride was added to each portion. The concentrations of potassium used were chosen on a logarithmic scale to give equal factors of increase or decrease.

Stable drugs were included in known concentration in the perfusion solution. For preliminary tests, and with unstable drugs, solutions of the substance in question were infused into the aorta of the perfused heart at a known rate over a period of 2–5 min. Concentrated stock solutions of the drugs used were made up on the day of the experiment, and further diluted as required. All solutions for infusion contained sufficient sodium chloride to bring their nominal osmolarity to 320 m-osmole; those of adrenaline also contained about 10 μN hydrochloric acid to prevent alkaline oxidation. In the initial experiments, designed to establish a suitable infusion rate of adrenaline, it was found that infusion of more than 100 μl./min of solutions with a pH below 4 led to the appearance of sinus arrhythmias in the perfused hearts. Hence the solutions of adrenaline for infusion were always prepared to have a pH above 4, and usually closer to 5. Provided that such solutions were kept enclosed, as in the infusion syringe, no oxidation was noticed; flushings from the infusion system, collected in an open beaker, rapidly developed a pink colour.

Coronary flows were measured where required by collecting the outflow of perfusion fluid from the heart for 30 sec periods, and converting to minute volumes.

Recording

The mechanical and electrical activity of the left ventricle were recorded on a Grass polygraph. The contractions of the ventricular muscle mass pulling towards the fixed aorta were measured using a force-displacement transducer connected to the apex through an insulating lever system. Fish hook recording electrodes of 0.4 mm diameter platinum wire were applied to the left ventricular wall and to the right atrium. Stainless steel clips attached to flexible wires were used as stimulating electrodes. One was clipped to the base of the left ventricle; it was necessary to be sure that the left auricle did not touch it, and that it did not occlude the left descending coronary artery. The second stimulating electrode was applied to the apex.

Channel 1 of the polygraph was used to monitor the mechanical activity. In most cases, a diastolic force of 50 g wt was applied; this gave almost maximal force. Ventricular action currents were picked up as the potential differences between the pair of recording electrodes, and these were then amplified and recorded on channel 2 of the polygraph. The amplified signal was also fed to one input of a dual-beam cathode ray oscilloscope and displayed; its R deflection was used to trigger the sweep in synchrony with the heart beat. A switch allowed the selection of whether ventricular or atrial electrical activity was applied to the input of the third polygraph channel, which further provided the option of recording either the normal electrogram (used for the atrial activity) or a *tachogram*, showing the frequency and regularity of beating; this was composed of a series of deflections linearly proportional to the time between successive input impulses. The tachogram was normally used.

Measurement of ventricular fibrillation thresholds

The method used for the measurement of ventricular fibrillation thresholds was based on that of van Tyn & MacLean (1961). An output pulse from the oscilloscope, synchronous with the start of the sweep, and thus with the R wave of the electrogram, was passed through a scale-of-two counter; on the start of every eighth sweep the output of the counter triggered a Grass model S4 stimulator. After the set delay, a 10 msec output pulse was generated and applied to the left ventricle. The voltage drop across a 100 Ω series resistor in one of the stimulating leads was applied to the second channel of the oscilloscope so that the *current* strength and timing in the cardiac cycle of the stimulus could be monitored. A 1μf capacitor was connected across the output terminals of the stimulator to produce a flat-topped pulse of stimulating current; without this, the initial peak was so sharp as to render accurate measurement of the stimulus strength impossible. With this instrumentation, 10 msec pulses of known strength could be applied to the left ventricle at determined intervals after every eighth ventricular depolarization.

Before determining a fibrillation threshold, the threshold current for diastolic extrasystoles was measured, using a delay from ventricular depolarization (taken as the peak of the R wave of the electrogram) of some 150% of the R-T interval of the electrogram as monitored on the oscilloscope. When this had been done, the stimulus pulse was then swept by 5 msec steps over a 50 msec period starting half-way through the R-T interval, and the current increased on successive sweeps from a starting strength close to the diastolic threshold value, using a standard logarithmic scale with 20 steps for a factor of 10, or 6 steps for a factor of 2. The number of steps on this scale by which the current was increased varied on different occasions: movement was by three steps at a time for the initial determinations, and by one step when an approximate threshold was known. The starting point of the sweep was always at least 10 msec earlier than the time when any extrasystolic response had been obtained on a previous sweep. During stimulation, the rise time constant of the electrogram amplifier was set at 0.1 sec, and the decay time constant at 0.2 sec. This reduced the amplitude of the pen record, but not that of the oscilloscope trace, and was done to damp the pen excursions due to stimulus artefacts.

These sweeps were repeated until ventricular fibrillation had been induced, and stimulation then stopped. The heart was allowed to continue fibrillating; if normal rhythm had not returned after 60 sec, defibrillation was effected by manually infusing 2 M potassium chloride solution (0.1–0.5 ml.) until complete electrical silence was seen on the ventricular electrogram, and the normal rhythm was then allowed to return as the excess potassium was washed out.

A stabilization period of at least 40 min was always allowed before the first fibrillation threshold was determined, and an interval of at least 15 min given between later determinations. Because of the transient nature of the changes reported by Hoffman *et al.* (1955), our determinations of the fibrillation threshold during an adrenaline infusion were started at the end of the first minute of the infusion.

Statistical methods

Standard statistical methods with frequent use of analysis of variance were employed to evaluate the results. Since the fibrillation thresholds showed a wide range, and yet only positive values were reasonable, it was most useful to consider them as being distributed lognormally (Gaddum, 1945). The calculations were therefore carried out on the logarithms of the thresholds, and the values of the arithmetic means of these later reconverted to the original scale of measurement giving geometric means on this. Where differences, standard deviations or standard errors were required, the values on the logarithmic scale were expressed as the corresponding factors on the original scale.

RESULTS

As had been expected, diastolic extrasystoles arising from stimuli applied shortly after the end of the ventricular action potential were of small amplitude but such responses

were made apparent by the increased amplitude of the next normal beat following the compensatory pause.

As the stimulus strength was increased, the delay from the R wave of the first pulse producing a simple extrasystolic response became less. This delay, marking the end of the *apparent refractory period*, would decrease gradually, but seldom became less than 60 msec in control hearts. On occasion during a single sweep, a response appeared, disappeared, and then returned to persist, as the delay to the stimulus was increased. This phenomenon usually marked a nearness of the fibrillation threshold. No attempts were made to measure the *effective refractory period*—that is, the delay between the reference ventricular depolarization and the response to the stimulus. Figure 1 (a) illustrates a typical extrasystolic response marking the end of the apparent refractory period at the stimulus strength shown.

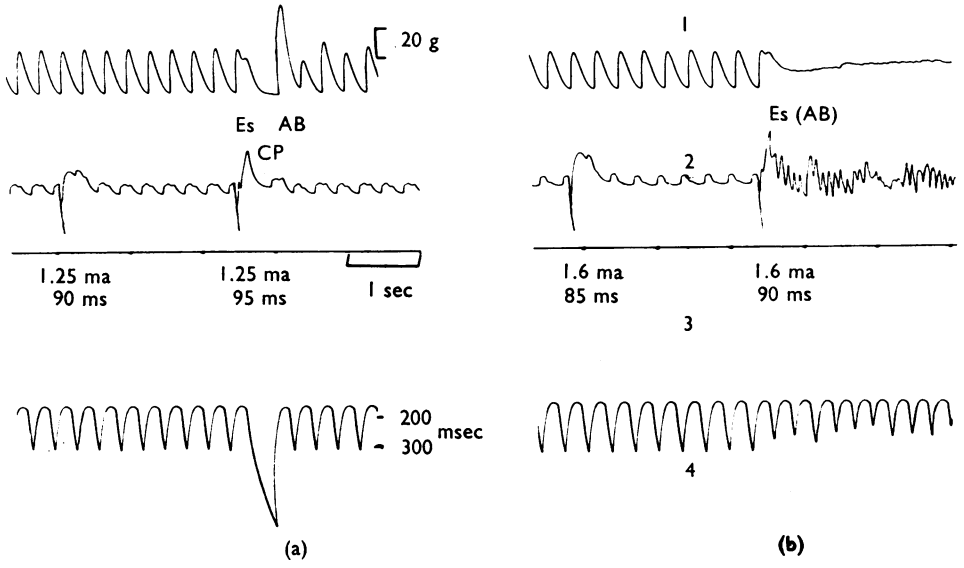

Fig. 1. Induction of ventricular fibrillation. Polygraph records show, reading down: ventricular force; ventricular electrogram, highly damped, with stimulus artefacts; time in seconds; current strength and delay from R wave; and cardiotachogram. Scales the same in (a) and (b). (a)=extrasystolic responses ES at subthreshold current, with augmented beat AB following compensatory pause CP. (b)=induction of ventricular fibrillation at threshold, with initial extrasystolic response and electrical response at expected time of next beat.

At a certain threshold stimulus strength, one pulse triggered ventricular fibrillation. This pulse might fall just inside or outside the apparent refractory period of the previous sweep. The onset of fibrillation, as shown in Fig. 1 (b), was sudden, and was apparent on the myogram as an irregular movement of extremely small amplitude, and on the electrogram as an irregular oscillatory rhythm. The tachogram also showed a typically irregular tracing. The most useful for normal diagnosis was the myogram. As can be seen in Fig. 1 (b), fibrillation started immediately following the extrasystolic response.

There was some indication of an electrical response at the expected time of the next normal beat. Once fibrillation had been induced and stimulation discontinued, the high frequency damping of the electrogram could be progressively lessened. The amplitude of the record increased as this was done, but its irregularity also increased.

Fibrillation, once induced, could persist indefinitely or could revert spontaneously to a normal rhythm. Early observations showed that spontaneous reversion normally occurred within 30 sec; hearts continuing to fibrillate beyond this would fibrillate for at least 15 min. As the force of contraction of the ventricles was markedly lower following a prolonged period of fibrillation, a 60 sec observation period was adopted. Hearts not reverting to normal rhythm within this time were recorded as *fibrillating persistently*; those reverting as *fibrillating non-persistently.*

Of the 174 non-persistent fibrillations observed, only 5 lasted more than 30 sec; 116 persisted for 5 sec or less, and 53 for between 5 and 30 sec. If short non-persistent fibrillation was obtained, further stimuli were applied. In some cases, a second stimulus of the same or greater current would produce a persistent fibrillation; in other cases, only non-persistent fibrillations were obtained, even with far stronger stimuli. If a persistent fibrillation was obtained at the same current as the non-persistent, the threshold was recorded as being for the persistent; if only at a higher current, then both thresholds—for non-persistent and for persistent fibrillation—were recorded.

The duration of a non-persistent fibrillation was of little significance: a heart could give long or short periods in response to successive effective stimuli with no regular pattern. On consecutive tests a given heart was consistent as to whether it gave non-persistent or persistent fibrillation, although there was a tendency for hearts giving only non-persistent fibrillations at an early stage to give persistent fibrillations later in the experiment. Conversion in the reverse direction was only seen once. Figure 2 shows typical records of non-persistent and persistent fibrillations.

The minimum duration of fibrillation accepted was arbitrarily set at 1 sec, as it was difficult to differentiate fibrillatory bursts of shorter duration from groups of three or four multiple extrasystoles.

Fibrillation threshold measurements were made for up to 7 hr after the start of a perfusion. The values determined in control experiments showed two main patterns: either they remained almost constant over the experimental period, or they gradually increased; this increase could level off after a few hours, and the value then remain stable. Because of the presence of the second pattern, all tests of the effects of treatments were made by comparing the threshold during treatment with those obtained both before and after.

Of 60 hearts tested, 59 fibrillated on the first trial, with thresholds ranging from 110 μa to 22.5 ma. These initial threshold values for each heart were analysed statistically, grouped according to whether *this* fibrillation was persistent or non-persistent. The results of the analysis are shown in Table 1.

Examination of the overall results showed that the hearts from all 8 rabbits weighing less than 1.75 kg gave non-persistent fibrillations on the initial test. This, in conjunction with the just significant difference in mean weights, supported the hypothesis that hearts from the smaller rabbits were more likely to show non-persistent rather than persistent

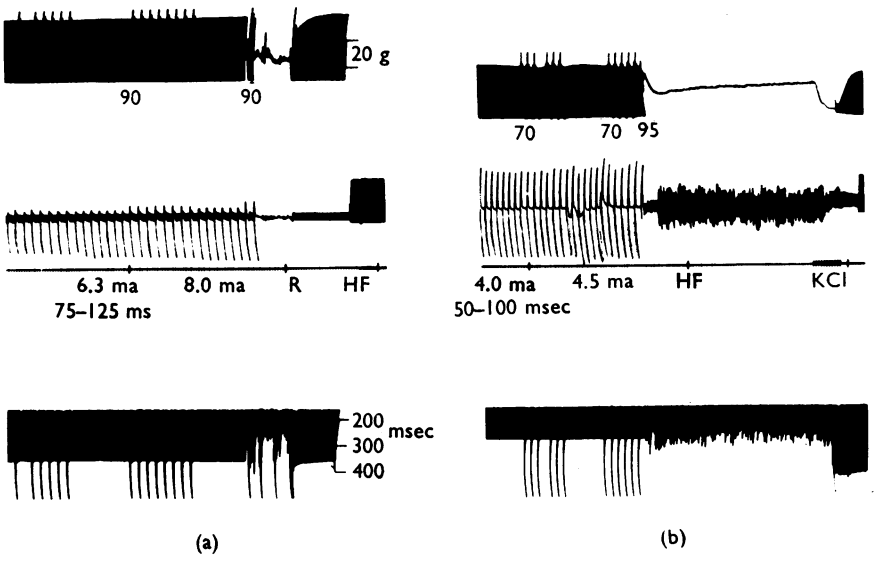

Fig. 2. Patterns of ventricular fibrillation. Polygraph records show, reading down: ventricular force; ventricular electrogram, highly damped, with stimulus artefacts; time in minutes and seconds; current strength and delay from R wave; the cardiotachogram. Scales the same in (a) and (b). (a) induction of non-persistent fibrillation, and spontaneous reversion at R; damping of electrogram removal at HF. (b) induction of persistent fibrillation, and defibrillation with KCL.

fibrillation. The correlation between weight and logarithm of the fibrillation threshold in the non-persistent group was positive; the test for difference in the mean thresholds was adjusted for the effects of this correlation. The significance of this difference indicated that for hearts from animals of the same weight the fibrillation threshold tended to be higher where only non-persistent fibrillation was observed.

The mean vulnerable time in these tests was 86 msec after the reference point of ventricular depolarization, with a standard deviation of 16 msec.

TABLE 1

STATISTICS OF INITIAL VENTRICULAR FIBRILLATION THRESHOLDS

	Non-persistent	Persistent	*P*: Difference in means
Number in class	24	35	
Mean weight	2·12 kg	2·36 kg	<0·05
SD	0·51	0·37	
Mean threshold	2·5 ma	1·5 ma	<0·02
SD (factor)	3·3×	2·6×	
P: correlation, weight/log threshold	<0·01	<0·3	

Effects of varying the potassium concentration

Potassium concentrations from one-half to twice normal (2.8 to 11.2 mM) were tested. Initially, attempts were made to find a potassium concentration just high enough to produce non-persistent rather than persistent fibrillation. In some cases the concentration needed was as high as 11.2 mM; such high concentrations were toxic in themselves, producing sinus arrhythmias and irregular beating.

The next step was to investigate the effects of variations in the potassium concentration on the value of the fibrillation threshold. Control thresholds were always determined before and after perfusion with the solutions of altered potassium concentration. The results were later plotted as in Fig. 3. A logarithmic scale was used for the values of

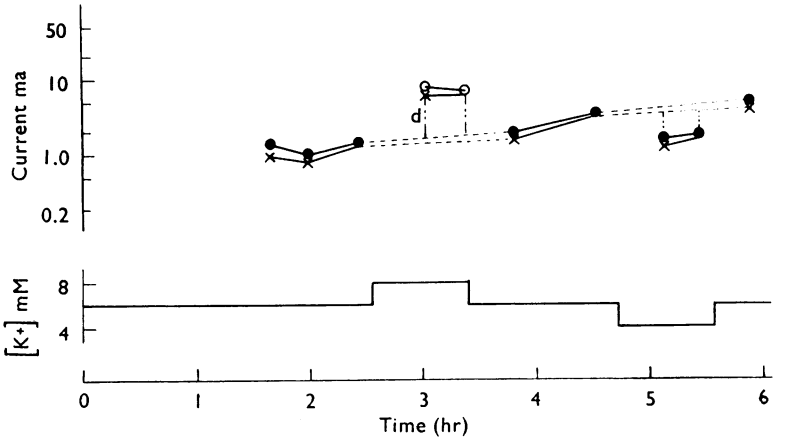

Fig. 3. Measurement of changes in the ventricular fibrillation threshold. Experiment with varied potassium concentrations. Ordinate=current in ma (log. scale); abscissa=elapsed time since excision of the heart. Crosses show the highest current at which no fibrillation was produced, and circles the lowest current at which fibrillation occurred. The highest non-fibrillating current is not shown by a symbol if it was only one step below the measured threshold. Persistence or non-persistence of the fibrillation is indicated by closed and open circles respectively. Dashed line indicates interpolation; broken line, as at d, the measured change in threshold. Potassium concentration of the perfusing fluid is shown on a log. scale.

the fibrillation thresholds, and these were plotted against the elapsed time since killing the animal. The change in fibrillation threshold resulting from the treatment was estimated as from the interpolated control values; where the threshold was not closely bracketed (to one step on the scale used), the minimum possible change was recorded. The changes were measured on the logarithmic scale, and the mean values on this scale converted to (geometric) mean ratios.

A concentration ratio of 1.4 was finally used to avoid secondary effects from highly unphysiological potassium levels: thus the low potassium concentration was 4 mM, and the high, 8 mM. Changes in the frequency and force of contraction occurred following the changes in potassium concentration, but were transient, and the original frequency and force were reattained after some 10 to 15 min. A half-hour stabilization

period was normally given before determining the first threshold under the new conditions. No significant changes in diastolic thresholds were seen. The fibrillation threshold was decreased at the low potassium concentration, and increased at the high. Such conversions between persistence and non-persistence of fibrillation as were observed were in the expected directions: non-persistence becoming persistence as the potassium level was lowered and *vice versa*. The overall results of the changes in the fibrillation threshold are shown in Table 2.

TABLE 2
EFFECTS OF TREATMENTS ON THE VENTRICULAR FIBRILLATION THRESHOLDS (VFT) OF ISOLATED HEARTS

Treatment	Tests (no.)	Mean change ± SE in $\log_1$ (VFT)	*P:* change from control	VFT change ratio*
Low potassium (4 mM)	10	−0·47±0·09	<0·001	1/3·0
High potassium (8 mM)	6	+0·61±0·02	<0·001	4·1
Adrenaline alone:				
8 n-mole/min	8	−0·11±0·04	=0·05	1/1·3
32 n-mole/min	8	+0·23±0·17	>0·2	1·7
Carbachol alone	6	+0·03±0·04	>0·5	1·1
Carbachol+adrenaline	4	−0·74±0·12	<0·01	1/5·5
Chloroform alone	3	+0·05±0·12	>0·7	1·1
Chloroform+adrenaline	4	−0·59±0·07	<0·01	1/3·9

* VFT change ratio: ratio of VFT under treatment to its control value; 1/3 denotes a reduction to one-third of the control level.

Direct effects of adrenaline infusions

The standard duration of an infusion was 5 min. With infusion rates of 1 n-mole/min (183 ng/min base) and above, a marked increase in frequency was seen, coupled with a transient increase in the force of contraction. The increase in frequency was maintained, and passed away quickly once the infusion was ended, but the force of contraction often fell below its previous control level during the infusion, and could take some 20 to 30 min afterwards to regain this original level. Increases in the coronary flow of the order of 15% were seen with moderate infusion rates (up to 50 n-mole/min); with higher rates, a marked decrease occurred. Both the frequency and the coronary flow had stabilized by the second minute of the infusion, and so the ½ min measurements of the coronary flow could be taken as representative samples.

As the infusion rate was increased, the beat interval tended to reach a minimum absolute value, similar for all hearts at about 240 msec, and unrelated to the control value. Figure 4 illustrates the overall effect: results were plotted only from experiments where two or more infusion rates were used, and those from two particular experiments at more extreme infusion rates are indicated separately on the graph. Both the infusion rates and the concentrations of adrenaline in the perfusion fluid (calculated on the basis of the stabilized coronary flow measured during the infusion) were recorded: as the scatter of the results was not decreased if the latter basis was used as abscissa, the former was chosen for ease of plotting and of statistical calculation.

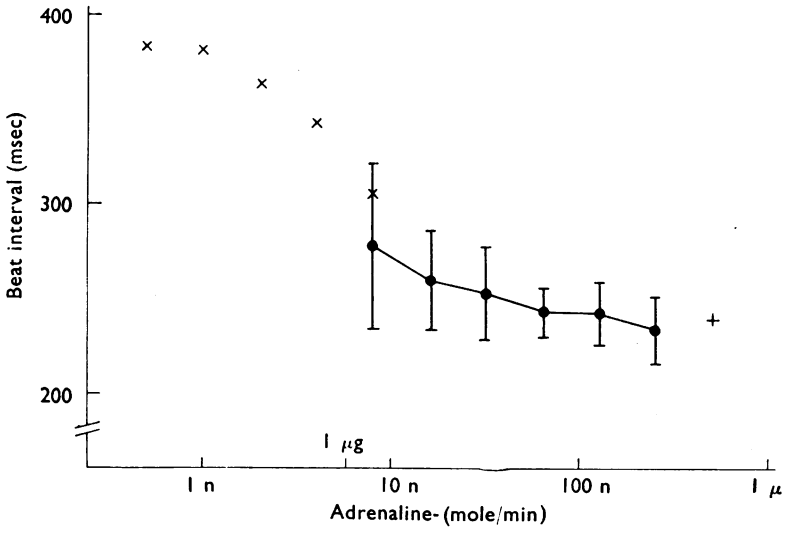

Fig. 4. Direct effects of adrenaline on frequency of heart heat. Mean ± SD of maximum absolute frequency reached (as time between beats). Abscissa = infusion rate of adrenaline in mole/min; rate of 1 μg/min also indicated. × = single values from experiment at low infusion rates. + = single value from another experiment.

With infusion rates of 8 n-mole/min and higher, a progressive series of pacemaker shifts appeared: these were well delineated by concurrent recording from the atrial and ventricular electrodes. Control observations showed a P-R interval of some 100 msec, and an R-T duration of about 150 msec. The first change seen was a deepening of the T wave and depression of the R-T segment until a single R-T deflection, some 100 msec long, was present. At this stage, the P-R interval had shortened slightly. Some isolated ventricular extrasystoles could appear, giving complexes of reversed polarity. The next phase was the transfer of the pacemaker to the A-V node, as shown by the almost simultaneous depolarization of the atria and ventricles, with no change in the contour of the ventricular electrogram. Then an idioventricular pacemaker developed: the ventricular complexes were of reversed polarity, but all of the same shape. The atria were now beating out of synchrony with the ventricles, and at a slightly lower frequency; both showed a beat interval of some 250 msec, a little greater than the minimum reached under the influence of the adrenaline. In a few occasions, ventricular fibrillation appeared suddenly; multifocal ventricular tachycardias were never seen. The onset of the arrhythmias due to the adrenaline often came after a short infusion had been stopped; this was particularly common for the idioventricular rhythms.

The effect of adrenaline on the fibrillation threshold

When the determinations of the fibrillation threshold were started, the change in frequency was almost complete. Most of the thresholds were determined within 3 min, and no correlation was seen between the size of any change found and the time of the determination.

The first tests were made with an adrenaline infusion rate of 8 n-mole/min (1.5 μg/min), which gave a mean concentration of 590 ± 50 (SE) nM in the perfusion fluid. This produced a marked acceleration to a mean beat interval of 280 ± 11 msec, and gave a minimum of arrhythmias; the worst seen was a coupled bigeminal rhythm similar to that described by Dresel, MacCannell & Nickerson (1960). As shown in Table 2, only small changes in the threshold were found with this infusion rate, the mean change from the control being two steps on the scale used.

As clear-cut changes were not obtained with 8 n-mole/min, the infusion rate was increased to 32 n-mole/min, with a mean concentration of 2.5 ± 0.25 μM. This occasionally produced runs of ventricular extrasystoles; the next higher rate would often give extended periods of ventricular tachycardia. At the increased infusion rate, the average effect was a rise in the threshold. Three experiments showed a slight fall, by four steps on the scale at most; one showed no change, and one, increases to 5–10 times the control value in three separate tests. These increases were the largest changes in threshold seen with adrenaline alone.

Although the fibrillation threshold was not significantly changed by the adrenaline infusion, the vulnerable time was. The control values were measured both before and after each infusion: no significant difference was seen within these pairs. The mean change due to the adrenaline was then calculated in each individual case, and these changes averaged. A reduction of 22 ± 3 msec was seen from the control values.

The effect of carbachol

Preliminary tests showed that with a carbachol concentration of 0.5–1 μM (90–180 ng/ml.) in the perfusing fluid, the beat interval was prolonged to more than one second, and yet the atria could still be driven at their original frequency. The P-R interval was not much altered by the carbachol when the atrial frequency was allowed to drop: if it was maintained at its original level by driving, then the interval from the stimulus artefact to ventricular depolarization would increase by some 20–30 msec from its original value near 100 msec. With a high enough carbachol concentration, a 2:1 block would appear; this could be abolished by decreasing the driving rate. As shown in Table 2, carbachol alone, even with the atrial driving, produced no significant change in the fibrillation threshold.

If, during perfusion with carbachol, adrenaline was then infused at 32 n-mole/min, a marked fall in the fibrillation threshold was seen. This occurred even in an experiment where infusion of the same amount of adrenaline before the carbachol was added produced almost no change. Table 2 again summarizes the results obtained.

The effect of chloroform

Enough chloroform was added to McEwen solution to give a final concentration of 1 mM (120 μg/ml.), and the mixture shaken to dissolve as much as possible. The spontaneous frequency and the force of contraction both decreased slightly when perfusion with this solution was started, but they returned to the control levels within 10 min. Again, chloroform did not of itself alter the fibrillation threshold, but when adrenaline was then infused, there was a considerable fall, as shown in Table 2.

DISCUSSION

In any investigation of factors affecting the induction or persistence of ventricular fibrillation, the first question arising is whether a reliable means of inducing fibrillation is available. It has been generally agreed by workers in this field (Wiggers & Wégria, 1940a ; Hoffman, Gorin, Wax, Siebens & Brooks, 1951 ; van Tyn & MacLean, 1961) that a single electricular stimulus of sufficient strength will produce fibrillation if and only if applied within the *vulnerable period*, which forms but a small part of the cardiac cycle. Increase of the stimulus strength above threshold will lengthen the vulnerable period, but only slightly (Hoffman *et al.*, 1951).

The essential truth of these statements has been confirmed for the isolated perfused rabbit heart in the present investigation. However, of the fibrillations produced in this way, only some 60% would persist indefinitely, the others reverting spontaneously to a normal rhythm. Such a proportion is reasonable in view of the results of MacWilliam (1887), Ferris, King, Spence & Williams (1936), Wiggers (1940) and others with hearts *in situ*.

Our control fibrillation thresholds showed a wider variation than those obtained on dog hearts *in situ* by Shumway, Johnson & Stish (1957) and van Tyn & MacLean (1961). This may have been due to the less physiological conditions of the isolated perfused heart ; a species difference is also possible: most of the data for hearts *in situ* are from dogs, while the present study was made using rabbits. Neither Wiggers & Wégria (1940b), nor Hoffman *et al.* (1951) give more than specimen figures, and it is not possible to determine what variations were present among their threshold values.

Potassium concentrations

Wide ranges of potassium concentration are possible only with isolated preparations, for *in vivo* both homeostatic and toxic effects limit the variation useable. Our failure to find changes in the beat frequency or force of contraction of the heart in the equilibrated state following changes of the potassium concentration from the normal 5.6 mM to 4 or 8 mM suggests that there is some homeostatic mechanism present at the level of the cardiac cells themselves, at least over this range of potassium concentration: this idea was supported by the fact that some changes did appear initially, but were transient. Thus the changes in fibrillation threshold found were not related to changes in frequency or force of contraction of the ventricles, nor to any variations in excitability (as measured by the diastolic threshold) which were also absent.

The highest potassium concentration tested was 11.2 mM ; at this level, only non-persistent fibrillations were found, but fibrillation could always be induced provided that a strong enough stimulus was used. Since a preliminary report of the present work appeared (MacConaill & Murnaghan, 1965), Lee, Richman & Visscher (1966) have agreed that perfusion with solutions of low potassium concentration predisposes to ventricular fibrillation, but they also reported spontaneous fibrillation with solutions of potassium concentration above 15 mM. This finding does not in fact conflict with our results, as we only investigated potassium concentrations up to 11.2 mM, and at this level marked disturbances in the spontaneous activity of the heart were already appearing.

Direct effect of adrenaline infusions

The tendency for the beat frequency to reach a fixed absolute level with high rates of adrenaline infusion was in concordance with the results of Trendelenburg (1960), who found the absolute change in the frequency of isolated atria to show less variation than the relative changes (fraction of control), and of Nickerson & Chan (1961), who had postulated an absolute ceiling, unrelated to the control value.

Although Dirken, Gevers, Heenstra & Huizing (1955) remarked on the induction of ventricular fibrillation in isolated hearts by the administration of large quantities of adrenaline, there is little recorded description of the arrhythmias with smaller doses. These followed the pattern of increasing severity described for dog hearts *in situ* (Dresel & Sutter, 1961), except that ventricular tachycardias were always monofocal, never multifocal; this was probably a species difference, due to the smaller size of the rabbit heart.

Effects on the fibrillation threshold

An attempt was made in our experiments to choose a dose of adrenaline which would produce the minimum arrhythmic effect of itself, and yet cause alterations in the fibrillation threshold. When this proved impossible, a higher dose was tried, and still no pronounced fall in threshold could be seen. The vulnerable period was indeed brought closer to ventricular depolarization, but this was most probably due to the shorter cardiac cycle produced with adrenaline acceleration.

The results of Goodford (1958) showing facilitation of ventricular fibrillation induction by adrenaline alone could not be duplicated. It is quite possible that the shifts in the vulnerable time and maximum follow frequency produced by the adrenaline could have made his fixed stimulation frequency more effective.

Our failure to obtain a marked fall in the ventricular fibrillation threshold with adrenaline alone is not really so surprising: Riker *et al.* (1955) have stressed the fact that their dose of adrenaline would produce many arrhythmias by itself, but not fibrillation; some sensitizing agent such as petroleum ether or chloroform (Levy, 1911) was necessary to give the latter response. The results obtained with adrenaline infusions when chloroform was present in the perfusion fluid confirmed this.

The fact that carbachol could also act as a sensitizer for an adrenaline-induced fall in fibrillation threshold is also of interest. Recent evidence has implicated vagal action in the production of idioventricular rhythms by adrenaline (Dresel, 1962). The mechanism proposed was that the vagal action would produce A-V nodal blocks, thus removing sinus dominance, and allowing the emergence of ventricular pacemakers; Mendez, Han & Moe (1964) have shown that vagal action will indeed produce such blocks at high heart rates, and such blocks were also seen by us.

The lack of effect of carbachol alone on the ventricular fibrillation threshold confirms the results of Armitage, Burn & Gunning (1957b), who found its only effect to be on the rate of stimulation they needed to produce fibrillation; this may be related to alterations of the vulnerable time.

SUMMARY

1. Ventricular fibrillation could be induced in isolated perfused rabbit hearts by the application of single 10 msec electrical stimuli during the vulnerable period of late

systole, and the threshold current could be measured. In 24 of the hearts so tested, the first fibrillation so induced reverted spontaneously to normal rhythm within a minute; in the other 35, the fibrillation persisted until arrested by excess potassium chloride. Later tests showed an increasing proportion of persistent fibrillations. The first fibrillation threshold measured ranged in different hearts from 100 μa to 22.5 ma, with a geometric mean value of 1.82 ma, the mean time of maximum vulnerability occurring some 85 msec after ventricular depolarization.

2. Raising the potassium concentration of the perfusion fluid from its normal 5.6 mM to 8 mM raised the fibrillation threshold to an average of four times its control value, while a reduction to 4 mM lowered it to a third of the control.

3. Infusion of adrenaline in amounts sufficient to produce maximal acceleration and mild arrhythmias failed on its own to produce marked changes in the fibrillation threshold, although the vulnerable period was brought significantly closer to ventricular depolarization. When carbachol or chloroform was included alone in the perfusion fluid in a concentration producing noticeable effects on the frequency, the fibrillation threshold was not altered: infusion of previously ineffective amounts of adrenaline now lowered this threshold to less than a quarter of its control value.

4. Administration of adrenaline alone to give concentrations up to 10 μM in the perfusion fluid produced A-V nodel rhythms, ventricular extrasystoles and tachycardia, and occasionally ventricular fibrillation.

This work was supported by a grant from the Medical Research Council of Canada and was carried out during the tenure of office of M. F. Murnaghan at the Department of Pharmacology, University of Ottawa.

REFERENCES

ARMITAGE, A. K., BURN, J. H. & GUNNING, A. J. (1957a). Ventricular fibrillation and ion transport. *Circulation Res.*, **5**, 98–104.

ARMITAGE, A. K., BURN, J. H. & GUNNING, A. J. (1957b). Ventricular fibrillation in the isolated rabbit heart. *Br. J. Pharmac. Chemother.*, **12**, 215–218.

DIRKEN, M. N. J., GEVERS, F., HEENSTRA, H. & HUIZING, E. H. (1955). A study of defibrillating agents on perfused rabbit hearts. *Circulation Res.*, **3**, 24–27.

DRESEL, P. E. (1962). Sites of vagal action in adrenaline-induced cardiac arrhythmias. *Can. J. Biochem. Physiol.*, **40**, 1655–1661.

DRESEL, P. E., MACCANNELL, K. L. & NICKERSON, M. (1960). Cardiac arrhythmias induced by minimal doses of epinephrine in cyclopropane-anaesthetized dogs. *Circulation Res.*, **8**, 948–955.

DRESEL, P. E. & SUTTER, M. C. (1961). Factors modifying cyclopropane-epinephrine cardiac arrhythmias. *Circulation Res.*, **9**, 1284–1290.

FERRIS, L. P., KING, B. G., SPENCE, P. W. & WILLIAMS, H. B. (1936). Effect of electric shock on the heart. Electron. Engng, **55**, 498–515.

GADDUM, J. H. (1945). Lognormal distributions. *Nature, Lond.*, **156**, 463–466.

GOODFORD, P. J. (1958). Metabolic factors and ventricular fibrillation. *Br. J. Pharmac. Chemother.*, **13**, 144–150.

HOFFMAN, B. F., GORIN, E. C., WAX, F. S., SIEBENS, A. A. & BROOKS, C. MCC. (1951). Vulnerability to fibrillation and the ventricular excitability curve. *Am. J. Physiol.*, **167**, 88–94.

HOFFMAN, B. F., SIEBENS, A. A., CRANEFIELD, P. F. & BROOKS, C. MCC. (1955). The effect of epinephrine and norepinephrine on ventricular vulnerability. *Circulation Res.*, **3**, 140–146.

LEE, Y. C. P., RICHMAN, H. G. & VISSCHER, M. B. (1966). $[Ca^{++}]$ and $[Ka^{+}]$ interrelations influencing mechanical and electrical events in cardiac activity. *Am. J. Physiol.*, **210**, 499–504.

LEVY, A. G. (1911). Sudden death under light chloroform anaesthesia. *J. Physiol., Lond.*, **42**, iii–vii.

MACCONAILL, M. & MURNAGHAN, M. F. (1965). Influence of various agents on the ventricular fibrillation threshold in the isolated perfused rabbit's heart. *J. Physiol., Lond.*, **179**, 55P–56P.

MCEWEN, L. M. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol., Lond.*, **131**, 678–689.

MACWILLIAM, J. A. (1887). Fibrillar contraction of the heart. *J. Physiol., Lond.*, **8**, 296–310.

MENDEZ, C., HAN, J. & MOE, G. K. (1964). A comparison of the effects of epinephrine and vagal stimulation upon the refractory periods of the A–V node and the bundle of His. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **248**, 99–116.

NICKERSON, M. & CHAN, G. C. M. (1961). Blockade of responses of isolated myocardium to epinephrine *J. Pharmac. exp. Ther.*, **133**, 186–191.

RIKER, W. F., DEPIERRE, F., ROBERTS, J., ROY, B. B. & REILLY, J. (1955). The epinephrine and hydrocarbon-epinephrine disturbance in the cat. *J. Pharmac. exp. Ther.*, **114**, 1–9.

SHUMWAY, N. E., JOHNSON, J. A. & STISH, R. J. (1957). The study of ventricular fibrillation by threshold determinations. *J. thorac. Surg.*, **34**, 643–653.

SZEKERES, L. & LENARD, G. (1960). Anderung der Flimmerbereitschaft der Vorhof- und Kammermuskulatur des isolierten Saugetierherzens nach Unterkuhlung und nach verschiedenen Pharmaka. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **238**, 454–459.

TRENDELENBURG, U. (1960). The action of histamine and 5-hydroxytryptamine on isolated mammalian atria. *J. Pharmac. exp. Ther.*, **130**, 450–460.

VAN TYN, R. A. & MACLEAN, L. D. (1961). Ventricular fibrillation threshold. *Am. J. Physiol.*, **201**, 457–461.

WÉGRIA, R. & NICKERSON, N. D. (1942). The effect of papaverine, epinephrine and quinidine on the fibrillation threshold of the mammalian ventricles. *J. Pharmac. exp. Ther.*, **75**, 50–57.

WIGGERS, C. J. (1940). The mechanism and nature of ventricular fibrillation. *Am. Heart J.*, **20**, 399–412.

WIGGERS, C. J, & WÉGRIA, R. (1940a). Ventricular fibrillation due to single localized induction and condenser shocks applied during the vulnerable period of ventricular systole. *Am. J. Physiol.*, **128**, 500–505.

WIGGERS, C. J. & WÉGRIA, R. (1940b). Quantitative measurement of the fibrillation thresholds of the mammalian ventricles with observations on the effect of procaine. *Am. J. Physiol.*, **131**, 296–308.