ANOXIA AND VENTRICULAR FIBRILLATION; WITH A SUMMARY OF EVIDENCE ON THE CAUSE OF FIBRILLATION

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Experiments are described showing that ventricular fibrillation is more readily produced in isolated rabbit hearts by electrical stimulation when the oxygen supply to the heart is reduced. This evidence completes investigations which have been made into factors affecting the production of fibrillation. These investigations have shown that factors which shorten the duration of the action potential, particularly those which cause the "plateau" to disappear, facilitate fibrillation, and those which lengthen the duration of the action potential prevent fibrillation. The reason for the length of the cardiac action potential may therefore be to prevent fibrillation. When the action potential is of normal length the fact that two adjacent fibres are out of phase does not matter; the one which is first to contract is not re-excited by the one which is second to contract, because at that moment the first is inexcitable. If the action potential is short, then the first may be already repolarized and may be excited by spread of excitation from the second. Factors which inhibit metabolism shorten the action potential.

When electrodes are attached to the ventricle of the isolated rabbit heart perfused through the aorta, a high rate of stimulation can then be applied for a given time. This causes the ventricles to fibrillate, as may be seen when the stimulation stops by using the electrodes as leads to an ECG. Ordinarily the fibrillation induced in this way continues only for 1 to 2 min. and a normal rhythm returns. However, under various conditions, such as in the presence of a low concentration of potassium or a high concentration of calcium, or in the presence of metabolic inhibitors, the fibrillation has been found to persist. The observations now to be described have been made to test the effect of a partial lack of oxygen.

METHODS

Rabbits were stunned and bled. The hearts were removed and attached to the apparatus for perfusion as already described (Armitage, Burn, and Gunning, 1957). The perfusion fluid contained (in mm.) NaCl 132, KCl 5.6, CaCl₂ 2.2, NaHCO₃ 2.5, NaH₂PO₄·2H₂O 0.92, dextrose 11.1, sucrose 13.1. The solution was saturated with 95% oxygen+5% CO₂ for the control observations, and with 47.5% oxygen, 47.5% nitrogen and 5% CO₂ for the rest. The temperature of the perfusing solution was 32° and was maintained within $\pm 0.5^\circ$, using the device of Saxby (1956). Hearts were

perfused at a pressure of 50 cm. water. Platinum fish-hook electrodes were inserted in the wall of the left ventricle, one at the apex and the other near the base. The stimuli were 1–2 mA. in strength, 2 msec. in duration and applied at a frequency of 20/sec. for 3 min. When stimulation was not being applied, the electrodes were used as leads to a Cossor electrocardiograph (model 1314); a record of the rhythm was obtained every 5 min. during fibrillation. The solutions saturated with the two gas mixtures were kept in separate flasks and perfused from separate Marriotte bottles.

When perfusion with any solution was begun, it was continued for at least 15 min. before stimulation was applied. When after stopping stimulation a normal rhythm was restored within 5 min., the result was recorded as "no fibrillation." When fibrillation persisted for more than 5 min., the result was recorded as "fibrillation."

RESULTS

Effect of Anoxia.—The following is an example of an experiment to determine the effect of reducing the oxygen supply. An isolated rabbit heart was perfused with a solution saturated with 95% oxygen for 15 min. and was then stimulated electrically for 3 min. When the stimulation stopped the normal rhythm was resumed at once. The perfusion fluid was then changed to one

saturated with 47.5% oxygen and 47.5% nitrogen, and after allowing this solution to perfuse for 20 min., stimulation was applied again. When the stimulation stopped, the heart was fibrillating and continued to do so for the next 15 min. Ice-cold saline was then poured over the heart and a normal rhythm returned. The perfusion fluid was changed back to the solution saturated with 95% oxygen. Stimulation was applied for 3 min. and when it was stopped a normal rhythm returned at once. The perfusion fluid was again changed to one saturated with 47.5% oxygen and 47.5% nitrogen, and after 20 min. the stimulation was applied for 3 min. Again the heart fibrillated when the stimulation was turned off, and continued to do so for the next 15 min.

This straightforward result was not obtained in all experiments, for in some the heart did not fibrillate when the solution contained only 47.5% oxygen. Use was then made of the observation (Armitage, Burn, and Gunning, 1957) that, when the potassium concentration was lowered, the proportion of hearts fibrillating was increased. The following experiment is an example.

The heart was first perfused with the usual solution containing 5.6 mm. potassium. When the perfusion fluid was saturated with 95% oxygen, stimulation did not cause fibrillation. When the perfusion fluid was changed to one saturated with 47.5% oxygen, stimulation caused fibrillation, but this continued for 2 min. only and then a normal rhythm returned. The heart was next perfused with a solution containing a lower potassium concentration, 4.5 mm., and saturated with 95% oxygen. Stimulation caused fibrillation which continued for 1 min. only before a normal rhythm returned. The heart was then perfused with a solution also containing 4.5 mm. potassium but saturated with 47.5% oxygen. Stimulation then caused fibrillation which continued for more than 15 min.

In all, 14 experiments were carried out, and, in 9, evidence was obtained that the lower oxygen saturation caused the heart to fibrillate more readily. In 4 experiments the results were similar to those in the first experiment described, and were the more convincing because, after observing that when a heart was perfused with a solution saturated with 47.5% oxygen, stimulation caused persistent fibrillation, it was possible to return to the solution saturated with 95% oxygen and show that stimulation caused only transient fibrillation. The results of the 9 experiments are set out in Table I.

In Expt. No. 1 of Table I the potassium concentration was 4.2 mm., and the result of the

TABLE I
EXPERIMENTS INDICATING THAT REDUCTION
OF OXYGEN PERCENTAGE FAVOURED VEN-
TRICULAR FIBRILLATION

The % oxygen represents the percentage of oxygen in the gas mixture with which the perfusion fluid was saturated.

Expt. No.	KCl (mm.)	No Fibrillation		Fibrillation	
		Trial	% Oxygen	Trial	% Oxygen
1	4.2	1	95	2	47.5
		3	95		
2	5.6	1	95	2	47.5
		3	95	4	47.5
3	5.6	1	95	2	47.5
		3	95		
4	5.6	1	95	2	47.5
		3	95		
5	5.6	1	95	2	47.5
		1	95		
6	5.6	2	47.5		
		3	95	4	47.5
7	5.6	1	95	2	47.5
		1	95	2	47.5
8	5.6	1	95	3	95.0
				2	47.5
9	5.6	1	95	3	95.0

first trial of stimulation, when the perfusion fluid was saturated with 95% oxygen, was that there was no fibrillation. The result of the second trial when the fluid was saturated with 47.5% oxygen was that persistent fibrillation developed. The result of the third trial was the same as that of the first.

There were 5 inconclusive experiments of which the following example is given. The heart was perfused with a solution containing 5.6 mm. potassium and was saturated with 95% oxygen. After stimulation fibrillation was transient. The heart was then perfused with the same solution saturated with 47.5% oxygen. When stimulation was applied, fibrillation was again transient. Perfusion was then continued with a solution containing a lower potassium concentration (4.4 mm.) and saturated with 95% oxygen. After stimulation this time, fibrillation was persistent. At the end of 15 min., fibrillation was stopped by pouring ice-cold saline over the heart. This result suggested that the reduction in potassium concentration had been too great, and a higher concentration (5.0 mm.) was tried. However, the heart fibrillated persistently when perfused with this solution saturated with 95% oxygen. Even

when a return was made to the original concentration of 5.6 mM. potassium, the heart perfused with the solution saturated with 95% oxygen fibrillated persistently after stimulation. Finally the heart was perfused with a solution containing 7.0 mM. potassium, but the heart did not fibrillate persistently after stimulation whether the solution was saturated with 95% or with 47.5% oxygen. With the lower saturation, however, it fibrillated for 4 min. There were 4 other similar inconclusive experiments which appeared to be due to an increasing tendency towards fibrillation as the duration of an experiment increased.

DISCUSSION

The observations described show that when the perfusion fluid was saturated with a gas mixture containing 47.5% oxygen instead of 95% oxygen, electrical stimulation of the ventricles more readily produced persistent fibrillation. Now oxygen lack has been shown to shorten the duration of the cardiac action potential. Trautwein, Gottstein, and Dudel (1954) found that, in cat papillary muscle stimulated at 140/min., the duration of the action potential was diminished by oxygen lack, the plateau being lost. This effect was reversed when oxygen was supplied. The work was confirmed in a later paper (Trautwein and Dudel, 1956). Webb and Hollander (1956) showed that the action potential of the rat atrium was diminished by anoxia; after 4 min. the diminution was 13%, after 10 min. it was 47% and after 17 min. it was 81%.

The evidence that the factors which facilitate fibrillation are those which shorten the action potential, and conversely that those which prevent fibrillation are those which lengthen it, is now considerable. Acetylcholine shortens the atrial action potential (Hoffman and Suckling, 1953) and facilitates atrial fibrillation in the dog (Burn, Vaughan Williams, and Walker, 1955). Acetylcholine has practically no effect on the ventricular action potential (Hoffman and Suckling, 1953) and has no effect on ventricular fibrillation (Armitage, Burn, and Gunning, 1957). Adrenaline lengthens the atrial action potential in the rat (Webb and Hollander, 1956), and prevents acetylcholine from causing atrial fibrillation (Burn, Gunning, and Walker, 1957).

Various metabolic inhibitors have been shown to shorten the action potential. Thus Macfarlane and Meares (1955) have shown that dinitrophenol reduces the plateau of depolarization in the atrium and ventricle of the frog to about one-third of its

normal length, and Kuschinsky, Lüllmann and Muscholl (1958) have shown that it reduces the duration of the action potential in the atrial muscle of the rat. Dinitrophenol was found to facilitate fibrillation in the rabbit ventricle (Armitage, Burn, and Gunning, 1957), and this effect was reversible. Another metabolic inhibitor is sodium azide, which Macfarlane (1956) found would reduce the duration of the action potential in the frog ventricle, and in keeping with this Goodford (1958) found that, in the presence of sodium azide, rapid stimulation of the ventricles led to persistent fibrillation as in the presence of dinitrophenol. The effect of sodium azide was reversible.

A third metabolic inhibitor is sodium monoiodoacetate. Lüllmann (1959) found that in its presence the duration of the action potential in the atria of the rat was shortened, and Goodford (1958) found that in its presence rapid stimulation of the ventricles caused fibrillation. This effect was not reversible.

A lack of glucose has also been shown to shorten the action potential in the rat atrium, the shortening becoming greater, the longer the time of glucose deprivation (Webb and Hollander, 1956). Goodford (1958) found that after perfusing rabbit hearts with glucose-free solution they continued to beat normally for periods of 2 hr. or more. By the end of that time some hearts began to fibrillate spontaneously, and, when the perfusion fluid was changed to one containing glucose, the fibrillation was arrested.

A factor which works in the opposite direction is a fall of temperature. The effect of lowering the temperature on the duration of the action potential was observed by Trautwein, Gottstein, and Dudel (1954) in cat papillary muscle. They found that in the range 38°–19°, the action potential, particularly the plateau, became longer as the temperature fell. The same observation was also made by Hollander and Webb (1955). Marshall (1957) observed the same change in the transmembrane potential of single fibres of the rabbit atrium; the duration of the action potential rose from 240 msec. to 400 msec. as the temperature fell from 27° to 18°. Now fibrillation can be arrested by pouring ice-cold saline over an isolated heart (Dirken, Gevers, Heemstra and Huizing, 1955). Beaulnes and Day (1957) working on isolated atria found that fibrillation produced at 37° was arrested when the temperature was lowered to 29° and began again when the temperature was raised. Similar observations were made by Goodford (1958) on the ventricles; he found that fibrillation

was arrested when the temperature was lowered from 37° to 32°.

Changes in calcium concentration affect the duration of the action potential. Hoffman and Suckling (1956) have found that the plateau was prolonged and the action potential was longer when the calcium concentration was low. When the calcium concentration was raised, repolarization occurred at a faster rate. Milton (1959) has shown that the proportion of hearts fibrillating in response to electrical stimulation is directly proportional to the calcium concentration in the perfusing fluid.

Changes in potassium concentration finally have a special interest, since there are two effects. Weidmann (1956) showed that large amounts of potassium shortened the action potential of the turtle ventricle. Vaughan Williams (1959) also found that in rabbit atria the action potential was shorter at 10 mM. concentration than at 5 mM. However, below 5 mM. the action potential also shortened as the concentration was reduced. Now, Milton (1959) found that when the potassium concentration was decreased below 5.6 mM. the proportion of hearts fibrillating increased. On the other hand, high concentrations of potassium are known to have a fibrillatory action. For example, Grumbach (1956) found that the injection of 1 ml. 0.77 M. potassium chloride into the fluid perfusing an isolated heart caused fibrillation if the heart had been exposed to adrenaline earlier.

Having thus seen several examples which point to a connexion between the duration of the action potential and fibrillation, we may ask what the connexion is. When the action potential in a fibre is short the fibre becomes re-excitabile very soon, and, if some nearby fibres are contracting, a spread of the excitation from them may cause the fibre to contract again.

We ordinarily suppose that an impulse from a pacemaker arrives at distant fibres simultaneously. But, when the impulses come quickly, the rate of conduction from the pacemaker to two distant but adjacent points may not be identical, and the impulse may not arrive simultaneously at the two fibres. One will then contract before the other contracts. Provided the action potential is long, the first fibre will be inexcitable when the second contracts. But if the action potential is short, and

the first fibre is rapidly repolarized, then this fibre will be stimulated to contract again by spread of excitation from the second fibre. There will be a local extrasystole.

Thus fibrillation could result from the spread of excitation from one fibre to another, when the fibres are out of phase and when the action potential is very short. The purpose of the long action potential of cardiac muscle seems to be to prevent this spread of excitation and so to prevent fibrillation. It is clear that the maintenance of the long action potential depends on a process which requires energy, since it is not maintained when there is lack of oxygen or glucose, or when metabolic inhibitors are present.

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