Brit. J. Pharmacol. (1957), **12**, 215.

VENTRICULAR FIBRILLATION IN THE ISOLATED RABBIT HEART

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(RECEIVED JANUARY 10, 1957)

Ventricular fibrillation has been produced in the isolated and perfused rabbit heart by stimulating electrically at a rate from 500 to 700/min. When the perfusion fluid contained normal amounts of sodium, potassium and calcium, the fibrillation persisted after the stimulation was stopped in about 40% of hearts. When the sodium was reduced to half, tonicity being maintained by sucrose or by choline chloride, persistent fibrillation was observed in 100% of hearts. The addition of eserine or of atropine or of carbachol did not alter the percentage of hearts in which fibrillation persisted. The antimalarial compounds chloroquine, mepacrine, and pyrimethamine arrested persistent fibrillation, restoring a normal rhythm.

Ventricular fibrillation can be produced in the Langendorff preparation of the rabbit heart by stimulating the ventricles at a high rate. At the end of stimulation the fibrillation continues in some hearts indefinitely, while in other hearts it stops after a minute or two. By testing a large group of hearts it is possible to determine the proportion of hearts in which fibrillation continues indefinitely. This proportion has been found to depend on the composition of the fluid perfusing the heart, and we have already described the effect of changes in the concentration of potassium ions and of calcium ions (Armitage, Burn and Gunning, 1957).

In this paper we describe the effects of (1) reducing the concentration of sodium ions, (2) adding carbachol, eserine or atropine to the perfusing fluid, and (3) the antimalarial substances chloroquine, mepacrine and pyrimethamine.

METHOD

Rabbit hearts were perfused with a solution recently described by McEwen (1956) containing NaCl 7.7 g., KCl 0.42 g., $CaCl_2$ 0.24 g., $NaHPO_4,2H_2O$ 0.143 g., $NaHCO_3$ 2.1 g., dextrose 2.0 g., sucrose 4.5 g., distilled water 1,000 ml. Thus the Na^+ concentration was 163 mM./l., the K^+ concentration was 5.6 mM./l., and the Ca^{++} concentration was 2.2 mM./l. The solution was saturated with O_2+5% CO_2 before being placed in the apparatus, and aerated again with the same mixture before going to the heart. The *p*H was 7.4. The temperature of the solution entering the heart was maintained constant at 37° C. at all rates of coronary flow by using the device described by Saxby (1956). For stimulation a pair of platinum electrodes was inserted in the ventricles through which rectangular pulses of 1 mA. strength and of 0.75 msec. duration could be applied at varying rates. A second pair of electrodes was inserted in the ventricles on the opposite side as leads to a Cossor electrocardiograph (model 1314). A mechanical record was also taken. When a heart was set up it was perfused for 30 min. with the solution described before changing to a modification of the solution which was to be tested. Stimulation was applied at a rate which was increased until fibrillation began. This usually occurred at a rate between 500 and 700/min. Stimulation was then continued for 5 min. When stimulation ceased the time for which fibrillation persisted was observed.

RESULTS

The Reduction of Sodium.—The solution already described was used to perfuse a series of 28 hearts. When fibrillation was established and stimulation was stopped, the fibrillation persisted during 30 min. in 11 hearts, while in 17 hearts there was spontaneous reversion to normal rhythm within 5 min. The amount of sodium chloride in the perfusing fluid was then reduced to one-half. In 13 experiments the tonicity was maintained by adding sucrose, and in each of these fibrillation continued for 30 min. Since the replacement by sucrose reduced not only sodium but also chloride, other experiments were carried out in which choline chloride was used instead of sucrose. The large amount of choline chloride depressed the rate and amplitude and caused A-V block in some hearts. The first two hearts, in which there was no block,

fibrillated for 30 min. after stimulation, while in the next two hearts block developed and stimulation failed to cause fibrillation. In the remaining five experiments 0.2 mg. atropine sulphate was injected into the cannula every 15 min., and stimulation then caused fibrillation which persisted for 30 min. in each experiment. The results are shown in Table I; they make it evident that the decrease in sodium increased the proportion of hearts fibrillating.

TABLE I

EFFECT OF REDUCTION IN Na^+ CONCENTRATION ON PROPORTION OF HEARTS FIBRILLATING

Na^+ Conc. mM./l.	Tonicity Maintained by Addition of	Proportion of Hearts Fibrillating for 30 min.
163	—	11/28
81·3	Sucrose	13/13
81·5	Choline chloride	7/7

Potassium Efflux.—In the previous experiments (Armitage, Burn and Gunning, 1957) it was observed that the efflux of potassium was increased during fibrillation whether the fibrillation was caused by reducing the potassium or by raising the calcium in the perfusion fluid. The estimation was made by the use of a Beckman flame photometer, the samples of perfusate being collected (1) while the rhythm was normal, (2) while the heart was fibrillating during stimulation, (3) while the heart was fibrillating after stimulation, and (4) after re-establishing normal rhythm by injecting 10 mg. KCl into the cannula. The mean result for samples (2) and (3) was then compared with that for samples (1) and (4).

Estimations of the potassium in the perfusate were made in the same way during perfusion with half the normal amount of sodium, and the results of five experiments are given in Table II. During fibrillation there was again a higher efflux of potassium ions in each experiment, the mean increase being 2.3%.

TABLE II

FIBRILLATION DURING PERFUSION WITH A SOLUTION CONTAINING HALF THE NORMAL SODIUM CONCENTRATION

K^+ Efflux (mg./l.)		
During Fibrillation	Before and After Fibrillation	% Increase During Fibrillation
224	217	3·0
212	211	0·5
226	218	3·7
223	217	2·8
225	222	1·3
Mean 222	217	2·3

Effect of Eserine and Atropine.—In the heart-lung preparation of the dog, electrical stimulation of the atria has been shown to produce auricular fibrillation when eserine was added to the blood (Burn, Vaughan Williams and Walker, 1955). Fibrillation produced in this way or by stimulation during the infusion of acetylcholine was arrested by atropine. Experiments were therefore carried out to see whether the presence of eserine increased the proportion of hearts with ventricular fibrillation and whether the presence of atropine diminished it. The results are given in Table III.

TABLE III

EFFECT OF ESERINE AND ATROPINE ON PROPORTION OF HEARTS FIBRILLATING

	Drug Conc. M.	Conc. of K^+ in Perfusion Fluid mM.	Proportion of Hearts Fibrillating
Control ..	—	5·6	11/28
Eserine ..	$1·2 \times 10^{-6}$	5·6	1/5
Control ..	—	2·8	10/11
Atropine	(See text)	2·8	13/15

In testing the effect of atropine, the solution used to perfuse the hearts contained half the normal potassium concentration, since this solution was previously found to cause fibrillation in 10 out of 11 hearts. In 10 of the experiments, 0.2 mg. atropine sulphate was injected into the cannula every 15 min., while in the other five experiments atropine sulphate was added to the perfusing solution in a concentration which varied from 5×10^{-8} to 10^{-6} g./ml. The results showed that eserine did not increase, and atropine did not diminish, the proportion of hearts fibrillating.

In two experiments the effect of injecting atropine on fibrillation during the stimulation was observed. In the first of these four injections of 0.5 mg. atropine, and in the second six injections of 0.5 mg. atropine arrested the fibrillation. In two other experiments in which the hearts had already fibrillated for more than 30 min. after stimulation, injection of atropine, 2.4 mg. and 1.0 mg. respectively, abolished fibrillation and restored a normal rhythm. These amounts were greatly in excess of that required to "atropinize" the heart.

The Effect of Carbachol.—In six experiments the effect of adding carbachol to the perfusing fluid was determined. The results are shown in Table IV. In none of the experiments did the presence of carbachol increase the tendency to fibrillate. In experiments 1 and 6 in which fibrillation was induced in the control period by driving at rates of 526 and 623 respectively, much higher rates of stimulation were required

TABLE IV
EFFECT OF CARBACHOL ON FIBRILLATION
Perfusion fluid contained 5.6 mM./l. K^+ and 1.1 mM./l. Ca^{++}

Expt.	Carbachol (g./ml.)	Rate of Stimulation to Induce Fibrillation		Duration of Fibrillation	
		Control	Carbachol	Control	Carbachol
1	5×10^{-7}	526	888	2·5 min.	More than 15 min.
2	2×10^{-6}	1,000	1,000	(No fibrillation)	(No fibrillation)
3	2×10^{-7}	1,000	1,000	,, ,,	,, ,,
4	10^{-7}	616	525	More than 15 min.	More than 15 min.
5	2×10^{-6}	1,000	1,000	(No fibrillation)	(No fibrillation)
6	10^{-7}	623	1,064	More than 15 min.	More than 15 min.

to cause fibrillation in the presence of carbachol. In experiments 4 and 6 in which fibrillation persisted in the control period, normal rhythm was restored by rapid cooling of the heart. This method of arresting fibrillation was described by Dirken, Gevers, Heemstra and Huizing (1955).

Effects of Chloroquine, Mepacrine and Pyrimethamine.—Armitage (1957) has observed that chloroquine, mepacrine and pyrimethamine have a quinidine-like action on the electrically-driven rabbit atria (Dawes, 1946). It therefore seemed likely that these substances would be effective in abolishing fibrillation, and their action was tested. Fig. 1 shows the ECG records from three experiments in which ventricular fibrillation had continued for more than 30 min. In the first experiment the fibrillation was arrested by chloroquine; in the second it was arrested by mepacrine, and in the third it was arrested by pyrimethamine. The concentrations of these substances in the perfusion fluid were respectively 2×10^{-5} g./ml., 10^{-5} g./ml., and 2×10^{-5} g./ml.

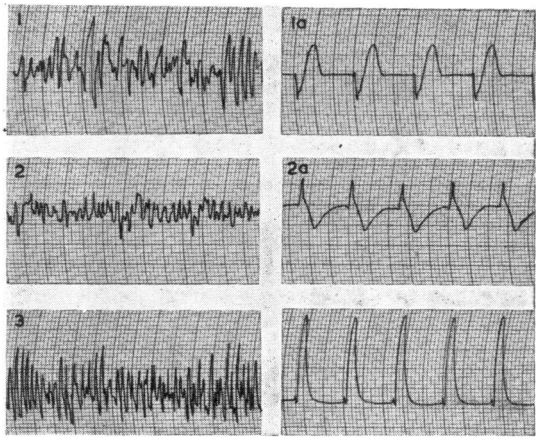

FIG. 1.—ECG records of isolated rabbit heart. (1) Shows ventricular fibrillation which continued for more than 30 min. (1a) Shows the normal rhythm restored within 4 min. by including chloroquine (2×10^{-5} g./ml.) in the perfusion fluid. (2) A different experiment; record as in (1). (2a) Shows the normal rhythm restored within 7 min. by including mepacrine (10^{-5} g./ml.) in the perfusion fluid. (3) A third experiment; record as in (1). (3a) Shows the normal rhythm restored by pyrimethamine (2×10^{-5} g./ml.) in 2 min.

DISCUSSION

The evidence that neither eserine, atropine nor carbachol affected the production of ventricular fibrillation is of interest because Hoffman and Suckling (1953) found that acetylcholine did not modify the ventricular action potential. By contrast these workers found that acetylcholine shortened the atrial action potential, and, since acetylcholine greatly facilitates atrial fibrillation, it appears that fibrillation in the atria or in the ventricles occurs only when the action potential is shortened. We now know that the shortening of the action potential in the atria is accompanied by an increase in the membrane conductance (Trautwein, Kuffler and Edwards, 1956) and by an increased permeability to potassium (Harris and Hutter, 1956).

Evidence is accumulating that in fibrillation there is an increased loss of potassium. In the isolated atria Holland, Burn, and Schümann (1957) have found that stimulation alone at increasing rates does not appreciably increase the potassium loss. If, however, the stimulation is applied in the presence of acetylcholine the potassium loss increases with the rate of stimulation until fibrillation occurs when the potassium loss exceeds a certain value (Holland *et al.*, 1957). Fibrillation in the ventricles is also attended by increased potassium loss, whether the fibrillation is caused by stimulation in a medium low in potassium, high in calcium, or, as has now been shown, low in sodium. The observations on potassium loss in the ventricles are, however, preliminary, and closer study is needed.

It is not easy to understand why a reduction of sodium to half should increase the tendency to fibrillation so greatly. One suggestion which can be made is that, when the sodium is reduced, less sodium enters the cell during contraction, and as a result the sodium pump has less work to do in extruding sodium. If less sodium is extruded, then less potassium may be replaced within the cell, if it is assumed that as in *Sepia* axons (Hodgkin and Keynes, 1954) the restoration of internal potassium is coupled with extrusion of sodium.

The action of chloroquine, mepacrine and pyrimethamine in arresting fibrillation may be

explained by effects which have recently been demonstrated. Armitage (1957) has shown that, when the isolated atria are exposed to the action of these substances, the contractions diminish in amplitude and frequency until they stop. They begin again when the external potassium concentration is lowered to one-half or one-quarter. This observation (also made with quinidine) suggests that these substances may make the membrane less permeable to potassium; the contractions may cease because the concentration gradient for potassium from inside to outside the cell is not then sufficient to allow exit of potassium. If, however, the external potassium is lowered, the potassium may once more travel out because the concentration gradient is raised. This explanation is supported by recent evidence that quinidine diminishes the uptake of K^+ by human red cells when cells kept at 0° C. for 3 to 5 days are then incubated at 37° C. (Kärki, Burn, and Burn, 1957).

This work was done by one of us (A. K. A.) during the tenure of a grant from the Wellcome Trustees.

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