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THE EFFECT OF ANTIFIBRILLATORY DRUGS ON THE CAT'S HEART *IN VIVO*

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It has been reported by Dews and Graham (1946), Dutta (1949), McCawley, Weston, and David (1951), and Schallek (1952) that several antihistamine compounds increase the relative refractory period of the isolated rabbit atrium as measured by the method of Dawes (1946). This method only indirectly measures changes in the refractory period produced by drugs. White and McCawley (1950) have reported that diphenhydramine is effective in preventing ventricular extrasystoles and tachycardia produced by cyclopropane and adrenaline in dogs. Burn (1950) and DiPalma and Schults (1950) suggested the clinical use of antihistamines as quinidine substitutes, and Dick and McCawley (1951) have demonstrated that they are effective against auricular fibrillation in man.

It was decided to investigate these drugs further in the hope of finding some qualitative difference in action between them and quinidine. By using the whole animal with the heart *in vivo* it was also thought that the results would be closer to clinical practice.

METHODS

Ventricular Fibrillation Produced by Chloroform and Adrenaline

Cats were anaesthetized with pentobarbitone sodium (30 mg./kg. intraperitoneally) and a tracheal cannula inserted. The carotid arterial blood pressure was recorded and injections were made into a jugular vein. Chloroform was given for 6 min. by allowing part of the cat's respiratory air flow to pass through a Wolff bottle containing chloroform, the flow being adjusted to produce the minimum fall in blood pressure. At the 5th min. 5 μg. of adrenaline was given intravenously and ventricular fibrillation usually started at once. The electrocardiogram was recorded from one of the three standard leads (usually lead II). The drug under investigation was given intravenously approximately 30 sec. after the start of ventricular fibrillation, and its ability to arrest the fibrillation was noted.

Ventricular Fibrillation Produced by Repetitive Electrical Stimuli

Cats were anaesthetized with pentobarbitone sodium (30 mg./kg. intraperitoneally) and artificial respiration was applied. The carotid arterial pressure was recorded and injections were made into a jugular vein. The chest was opened, the pericardium slit, and a bipolar silver/silver chloride stimulating electrode was placed on the surface of one ventricle. The voltage drop across a 100 Ω resistor in series with the stimulating electrodes was measured on a calibrated oscilloscope. The heart was stimulated at a rate of 45/sec. with rectangular current pulses of 800 μsec. duration. The minimal current required to produce ventricular fibrillation, as shown by the electrocardiogram, was observed three times and then the drug was given. The power of the drug to raise the threshold current necessary to produce ventricular fibrillation was used as a test of anti-arrhythmic activity.

Determination of Refractory Period, Excitability, and Atrio-ventricular Conduction Time of the Intact Cat Heart in vivo *(Single Shock Method)*

The method used resembles that of Suckling, Brooks, Orias, Gilbert, and Siebens (1950), but instead of the heart being artificially driven it is allowed to beat normally. The disadvantage of this—that changes in rate produced by a drug will produce changes in relative and absolute refractory periods—has to be weighed against the fact that it is the total effect of the drug that is the important thing to measure.

Cats were anaesthetized with pentobarbitone sodium (30 mg./kg. intraperitoneally), a tracheal cannula was inserted, and artificial respiration applied. The chest was opened and both phrenic nerves divided to avoid movements of the diaphragm. The pericardium was opened and screened silver recording electrodes, 1 mm. in diameter, were placed on the surface of the left atrium and of each ventricle. The amplified action potentials were displayed on a double beam oscilloscope. One of these action potentials, from either atrium or ventricle, was used to generate a test pulse, after a variable delay. The provision of this stimulus, which could be applied to the heart at any time interval after the previous ventricular action potential, enabled measurements of refractory period to be made with comparative ease.

The heart was stimulated through a silver/silver chloride electrode, an indifferent electrode being placed in the right intercostal muscles and connected to earth through a 1,000 Ω resistor. The cat was also earthed by its brass tracheal cannula. The fraction of current

passing through the indifferent electrode was measured from the voltage drop recorded on a calibrated oscilloscope. Preliminary investigations showed that this fraction bore a constant relation to the total current passing through the animal under the conditions used.

The heart rate, refractory period, ventricular conduction time, the duration of the ventricular action potential, atrio-ventricular conduction time, blood pressure, rectal temperature and strength-duration curves were determined on several occasions until steady measurements were obtained before the drug under test was given.

The total refractory period was determined by measuring the shortest time after the beginning of the previous action potential at which the minimal strength of shock of 0.065 msec. duration produced a premature action potential. The absolute refractory period was determined by finding the minimal time after the start of the last beat at which a supramaximal shock produced a premature action potential. Atrio-ventricular conduction time was taken as the time interval between the peak of the atrial and ventricular action potentials. The time interval between the peak of the ventricular action potential picked up by local spread to the atrial electrode and that picked up directly by the ventricular electrode was also recorded as an indirect index of conduction velocity through ventricular muscle or the conducting system.

Results

Fibrillation from Chloroform and Adrenaline.—Preliminary observations with procaine amide showed that it was effective in arresting ventricular fibrillation induced by adrenaline in cats under light chloroform anaesthesia. However, the method did not give reproducible results in six experiments.

Fibrillation from Repetitive Electrical Stimulation.—The minimal current strength required to induce ventricular fibrillation was usually increased by procaine amide in nine cats. However, as the normal threshold was in the region of 1–6 mA and varied considerably, it was necessary to produce a large change to be sure that a particular drug was effective. This increase would be in the region of 8–20 mA, depending on the initial threshold; at these current strengths, using bipolar stimulating electrodes, the local current density was so high that burning of the heart resulted. By using a unipolar electrode the current needed to produce ventricular fibrillation could be reduced. However, the current spread was then greater, and, especially at the high strengths required to produce fibrillation after the administration of a drug, caused widespread nerve and muscle excitation, the whole animal twitching violently.

Ventricular Tachycardia Produced by Electrical Stimulation.—This method was tried because ventricular tachycardia occurred at a much lower current strength than did fibrillation, and it was thought that it would be possible to detect a graded increase in this threshold without using current strengths which caused burning or widespread tissue excitation. Although the blood pressure fell considerably during the initiation of the ventricular tachycardia it did not drop to zero as with ventricular fibrillation.

The results with this method on 15 cats were even more erratic than with the previous methods. Sometimes a rise was detected in the tachycardia threshold, with a later return to normal; but at other times the only effect noted was a slowing of the rate of the ventricular tachycardia (see Fig. 1). Mepyramine,

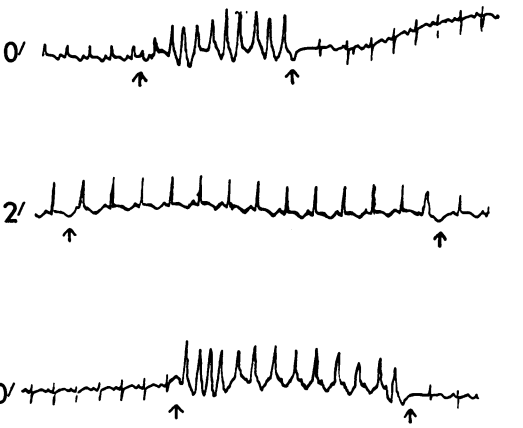

FIG. 1.—Effect of promethazine on the threshold to ventricular tachycardia induced by direct electrical stimulation of the ventricle in the cat. Upper electrocardiogram trace (0 min.) shows a burst of ventricular tachycardia during electrical stimulation of constant strength. 5 mg./kg. of promethazine given at 1 min. 15 sec. Centre trace at 2 min. shows absence of ventricular tachycardia during stimulation of the same strength as in the upper trace. Lower trace at 20 min. shows return of threshold to normal with development of ventricular tachycardia in response to electrical stimulation of the same intensity as in the upper trace. The additional effect of the drug in slowing the rate of ventricular tachycardia is also seen. Beginning and end of period of stimulation indicated by arrows in each trace. Electrocardiogram: lead II (right arm, left leg). (Tracing retouched.)

diphenhydramine, procaine amide, and quinidine sometimes caused an increase of the tachycardia threshold, but the only consistent effect of these drugs was to cause slowing of the rate of ventricular tachycardia. It must be noted that the tachycardia lasted only for the duration of stimulation. The ventricles were therefore merely being driven by the repetitive stimuli, the conditions being different from that of ventricular fibrillation, which is self-supporting.

An alteration in ventricular tachycardia threshold could result from many changes, such as an increase in relative refractory period, which would mean that most of the stimuli would fall in this period so that the threshold would appear to increase. This is indicated by the slower rate of the ventricular

tachycardia when induced after a particular drug, the effect being comparable with that seen in the isolated rabbit atrium (Dawes, 1946). A decrease in the excitability of the ventricular muscle could also result in a similar change.

To demonstrate that the factors responsible for a change in fibrillation threshold were different from those for a change in tachycardia threshold, the two thresholds were measured in the same animal before and after procaine amide. The results on five cats, of which a good example is to be seen in Table I, show that whereas the fibrillation threshold was increased beyond the scope of the stimulator after procaine amide, the ventricular tachycardia threshold remained unchanged.

TABLE I

TYPICAL CHANGES PRODUCED BY PROCAINE AMIDE IN VENTRICULAR TACHYCARDIA AND FIBRILLATION THRESHOLDS TO DIRECT ELECTRICAL STIMULATION OF VENTRICLES IN THE CAT

Time (min.)	Ventricular Fibrillation Threshold (mA)	Ventricular Tachycardia Threshold (mA)
0	3·5	0·22
3	3·0	0·25
6	3·2	0·35
7	Procaine amide 25 mg./kg. intravenously	
9	>20	0·28
12	>20	Not tested
15	>20	"
64	17	0·28"

Instead, therefore, of measuring the increase in the threshold for the development of these arrhythmias, the refractory period, atrio-ventricular conduction time, and excitability were measured directly.

Single Shock Method

Experiments were performed in 41 cats, each cat receiving 1–4 drugs during the course of the experiment. Each drug was the first to be tested in at least two cats. All measurements were allowed to return to steady levels before a further drug was given, and the drug tested was given at least twice to each cat.

Quinidine, 5 mg./kg., was tested in 10 cats, and in all of these it was the first drug to be given. The effects are shown in Table II.

Nine antihistamine compounds were tested, in a dose of 5 mg./kg.—mepyramine, promethazine, pyribenzamine, chlorprophenpyridamine, chlorcyclizine, phenindamine, antazoline, and diphenhydramine. 3300 R.P. was also examined; though chemically similar to promethazine, it has only slight antihistamine activity. All these substances had, to a variable degree, the property of increasing the

TABLE II

MEAN RESULTS OF THE EFFECT OF 5 MG./KG. QUINIDINE SULPHATE, MEPYRAMINE, PHENINDAMINE, AND 3300 R.P. INTRAVENOUSLY IN CATS

	% Increase Over Control Readings			
	Quinidine	Mepyramine	Phenindamine	3300 R.P.
	Mean±S.D.	Mean±S.D.	Mean±S.D.	Mean±S.D.
Interval between two ventricular systoles..	13·1*	24·2±16·7	36·1±20·5	7·6± 4·6
Total refractory period ..	18·0±12·6	22·7± 5·8	45·2±22·4	12·8± 8·3
Relative refractory period	25·1*	25·3*	103·9*	16·8±15·6
Absolute refractory period..	20·6±10·9	25·0±10·6	46·4±27·6	9·2± 8·8
Atrio-ventricular conduction time ..	16·7±11·3	15·3± 8·3	59·6*	19·7±13·9
Conduction velocity in ventricle ..	Increased	Increased	Increased	Increased
No. of cats ..	10	5	5	4

* S.D. greater than mean.

total, relative, and absolute refractory periods, the duration of the action potential, and the conduction velocity of the ventricular muscle. They also increased atrio-ventricular conduction time and decreased the heart rate. The effects varied considerably from cat to cat and it is possible only to give an approximate comparison with quinidine: although quinidine sometimes appeared to have more effect on the relative refractory period than did the antihistamines this was not always so. Chlorcyclizine and phenindamine appeared more active than quinidine. Mepyramine, promethazine, diphenhydramine, pyribenzamine, and antazoline were next in order of activity—comparable with that of quinidine—whereas chlorprophenpyridamine and 3300 R.P. showed little activity. Table II gives representative examples of the effect of each of these groups, compared with that of quinidine. The large standard deviations give some indication of how variable the results were.

The duration of action of all these drugs was usually very short—rarely longer than 10 min. All generally produced, like quinidine, a profound fall in blood pressure lasting 15 to 30 sec. The typical changes produced are shown in Table II.

Excitability.—It proved difficult to obtain consistent measurements of the strength-duration curve. Various types of stimulating electrodes were used. Bipolar electrodes gave very erratic current readings, the interelectrode resistance changing considerably according to the state of the heart. The variation was reduced by using a unipolar stimulating electrode on the heart and an indifferent electrode inserted into the intercostal muscles. Another difficulty in interpreting apparent changes in the

strength-duration curve was that drugs often increased the relative refractory period so that it continued until the next beat.

However, quinidine and the antihistamines did appear to increase the threshold to the shorter duration shocks; those of long duration were less affected. This increase was always accompanied by changes in the refractory period and conduction time. A typical example is shown in Fig. 2. There appeared to be no qualitative difference between quinidine, the antihistamines, and procaine amide in these effects.

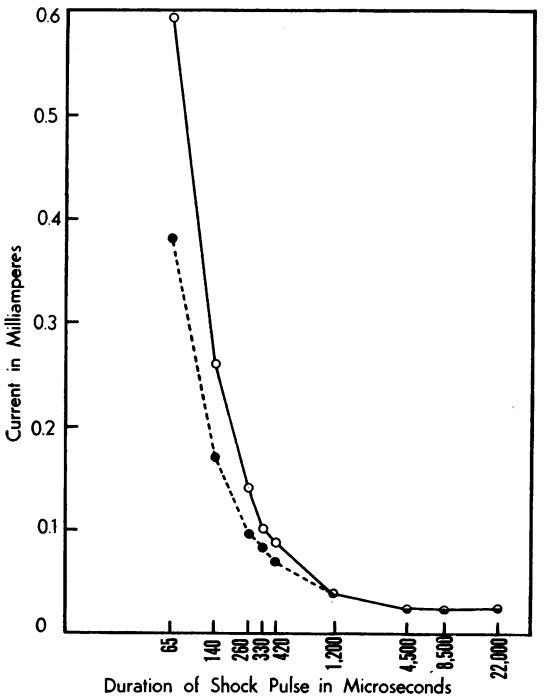

FIG. 2.—Typical strength-duration curve of ventricular muscle of the cat heart *in situ*. Abscissa: logarithm of the duration of the stimulating square wave shock in microseconds. Ordinate: minimal current in mA required for excitation. Interrupted curve before, and continuous curve after, 5 mg./kg. of diphenhydramine intravenously. The effect of the drug is to increase the threshold to short rather than to long duration shocks.

Other drugs were tested on a few cats. Procaine amide (25–50 mg./kg. in four cats), synephrine (20 mg./kg. in one cat), tolazoline (2.5 mg./kg. in one cat), and cocaine hydrochloride (3.0 mg./kg. in one cat) all cause changes resembling those produced by quinidine. Digoxin in one cat in a total dose of 0.15 mg./kg. produced no change in the strength-duration curve although this dose of digoxin eventually killed the cat. In one cat, aminophylline, 15 mg./kg., had no effect. Relatively large doses of caffeine (one cat, 20 mg./kg.) slightly reduced the absolute and relative refractory periods and slightly increased the heart rate.

A group of six cats treated for two weeks with thyroxine-sodium (0.1 mg. daily by mouth) showed no significant difference from normal cats. However, the normal range is wide.

Fibrillation Threshold.—No evidence could be found for the existence of a vulnerable period in the cat ventricular muscle, as described by de Boer (1921) in the frog ventricle and Andrus and Carter (1930), Wégria and Wiggers (1940), and Wiggers and Wégria (1939; 1940) in the dog. Ventricular fibrillation was not obtained with single shocks but was produced by repetitive stimuli of the same intensity. However, current strengths of not more than 5 mA were used, and the authors quoted used 10–20 mA. Even with 5 mA single shocks there was gross current spread as shown by extensive nerve and muscle excitation. It may be that the effect of single shocks which cause ventricular fibrillation is to produce prolonged depolarization of the ventricular muscle. Such a depolarization might favour the development of irregular recovery of excitability and re-entry of the propagated disturbance.

DISCUSSION

The method used for causing ventricular fibrillation by adrenaline during light chloroform anaesthesia produced very variable results. The circulatory failure which resulted must have led to anoxia of the cardiac muscle, and one cannot be certain whether the action of a drug in stopping the fibrillation is by way of an antiadrenaline effect, or by a direct action on the heart itself. Moreover, the mode of action of chloroform and adrenaline in producing ventricular fibrillation is unknown. Hence the use of this method is empirical and of doubtful value in screening possible antifibrillatory agents. It also involves the use of large numbers of cats.

Somewhat similar conclusions apply to the method which uses repetitive electrical stimuli to induce fibrillation. This method may cause burning of the ventricular muscle and extensive spread of excitation. The use of ventricular tachycardia as an end point is also likely to give false information, for the arrhythmia induced is not self-supporting.

The single shock method in which changes of refractory period, conduction time, and strength-duration curve are measured in the normally beating heart gives more information than the other methods and is the main justification for its use. The conduction velocity in ventricular muscle, as measured by two electrodes on the muscle itself, was so high

that only a few milliseconds were required for the action potential to invade the whole of the ventricles; although this was increased by the quinidine-like agents tested, it was still only a fraction of the time occupied by the relative and absolute refractory periods (200 msec. approximately). It is therefore difficult to imagine that this increase will counteract the effect of an increase in the refractory period on a hypothetical circus movement.

The measurements of excitability posed some interesting problems. Thus, one was in doubt as to whether a change in the strength-duration curve as measured was the combined result of many changes in the muscle or was due merely to an alteration in the magnitude of the resting membrane potential or its potential for threshold depolarization. When using external electrodes there may be changes in an equivalent impedance in series or in parallel with the membrane and electrode unconnected with changes in the muscle itself. Burgen and Terroux (1953) showed that, although change in the strength-duration curve indicated an increase in excitability of the isolated cat's atrium after acetylcholine, the membrane potential was actually increased. This might be due to a change in the rate of increase of sodium conductance after threshold depolarization. A slowing of the rate of increase in sodium conductance might be accompanied by a reduction in the rate of collapse of membrane potential, and hence also a reduction in the velocity of conduction of the excitatory wave. It seems possible then that excitability and conduction velocity, and perhaps some other variables, may be fundamentally related.

Some confusion surrounds the meaning of the term " excitability." Clinically, an increase or decrease in " excitability " is often used to describe an increase or decrease in the predisposition of the heart to the development of arrhythmias. Thus adrenaline and digitalis are said to increase " excitability " of the heart and quinidine to decrease it. That a change in " excitability " as meant above is the result of a change in the electrical excitability as determined by a strength-duration curve does not necessarily follow. Large doses of digoxin, which are said to increase excitability of the cardiac muscle, produced no change in the strength-duration curve in the present work.

The results of the single shock method did not reveal any qualitative differences in action between quinidine and the antihistamines. The doses of antihistamine compounds needed to cause comparable effects in the cat were of the same size as those used for quinidine. The results for procaine amide differ from those observed by Woske, Belford, Fastier, and Brooks (1952), who found it did not increase the refractory period of ventricular muscle very much but greatly decreased electrical excitability.

It seems unlikely that any fundamental advance will be made in our understanding of the mode of action of these drugs upon the heart until their effect upon individual cardiac muscle fibres has been examined more thoroughly.

Summary

1. The quinidine-like or antifibrillatory activity of drugs on the cat's heart *in vivo* has been examined by four methods.

2. Quinidine, several antihistamine compounds, procaine amide, and other drugs had qualitatively similar actions on refractory period, conduction velocity, electrical excitability, and atrio-ventricular conduction time. Chlorcyclizine and phenindamine appeared to be slightly more active than quinidine.

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