Brit. J. Pharmacol. (1961), 17, 424–432.

A COMPARISON OF TESTS FOR ANTIFIBRILLATORY ACTION

BY

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(*Received August* 4, 1961)

The object of the experiments was twofold: first, to choose the most satisfactory test for antifibrillatory action; secondly, to place several drugs with reputed antifibrillatory activity in an order of potency as a preliminary to investigating their mode of action. Measurements were made on isolated rabbit atria at 34° C of (1) the maximum driving frequency the atria would follow, (2) conduction velocity, (3) contractions, and of the threshold for the production of (4) extrasystoles, (5) flutter and (6) fibrillation. Log dose-response curves were plotted for quinidine, papaverine, procaine, dibenamine and procaine amide. The maximum frequency test and fibrillation threshold test gave similar results with all the drugs, and the results gave the order quinidine 1.0, procaine 0.53, Dibenamine 0.47, papaverine 0.43 and procainamide 0.26. Thresholds for extrasystoles and flutter were much more variable. The regressions relating changes in conduction velocity and contraction to log dose were different from those for maximum driving frequency and fibrillation threshold for procaine, papaverine and dibenamine, but the regressions for quinidine and procaine amide were nearly parallel in all tests. Serpajmaline contained a substance with antifibrillatory activity as great as that of quinidine and with no greater depressant action on contractions.

There is still no satisfactory explanation for the phenomenon of fibrillation, which makes the interpretation of the mode of action of antifibrillatory drugs difficult. Although it has been established that Lewis's hypothesis of a circular sequence of excitation can no longer be maintained in its original form (Scherf & Schott, 1953; Prinzmetal, Corday, Brill, Oblath & Kruger, 1952), and that flutter and fibrillation can originate from an ectopic focus, it remains to be discovered why a particular region of heart muscle should suddenly become an ectopic pacemaker, and why uncoordinated activity should spread from such a focus over the whole of the atria or ventricles. It is a fact, however, that fibrillation can be precipitated by a variety of methods, including the focal injection of aconitine (Scherf, 1947), the combined action of cholinergic drugs and electrical stimulation (Burn, Vaughan Williams & Walker, 1955), or by suitably timed electrical stimulation alone (Dipalma & Schults, 1950; Brooks, Hoffmann, Suckling & Orias, 1955; Szekeres & Lénárd, 1960), and that fibrillation, both clinical and experimental, can be stopped or prevented by a number of substances of quite different chemical structure.

Compounds with allegedly antifibrillatory action have usually been compared (Dawes, 1946) with quinidine, as the parent antifibrillatory drug. Quinidine was at first claimed to prolong the absolute refractory period of cardiac muscle (Lewis, Drury, Iliescu & Wedd, 1921) and then shown not to do so (Love, 1926). Yet there

is no doubt that quinidine reduces the frequency at which cardiac muscle can be induced to follow strong electrical stimuli, that is, it increases the "effective" refractory period (Lewis & Drury, 1926), and also increases the threshold to stimulation by electrical current or an ectopic focus (Scherf & Schott, 1953; Prinzmetal, Corday, Brill, Oblath & Kruger, 1952).

Authors still do not agree how antifibrillatory drugs work, however (Vaughan Williams, 1958b; West & Amory, 1960; Burn, 1960). Any hypothesis concerning their mode of action would obviously be strengthened if it could be shown that all drugs with known antifibrillatory effects had certain properties in common. It would first be necessary, however, to define what constituted an antifibrillatory drug. Many different tests for antifibrillatory action have been described, but there has not been, so far as we know, any previous attempt to compare them quantitatively. The object of the experiments described here was to place several substances, with reputed antifibrillatory activity but of different chemical structure, in an order of potency by comparing their relative performance in several different tests. Any other action they might have in common, for example, a depression of the rate of rise of the action potential in the absence of any fall in resting potential or prolongation of repolarization, as has been described by Szekeres & Vaughan Williams (1961), would provide some evidence for the relevance of this effect to their antifibrillatory action.

METHODS

Isolated rabbit atria were suspended horizontally in a water-jacketed bath, the temperature of which was kept at 34° C, and were supplied with modified Locke solution as previously described (Vaughan Williams, 1958a; Szekeres & Vaughan Williams, 1961). Re-oxygenation of the bath fluid was carried out in a subsidiary rapid circulation, fluid being continuously withdrawn, oxygenated and returned to the bath. Contractions were recorded by an RCA 5734 transducer, and displayed on one beam of a Dumont 322 oscilloscope. Action potentials for the measurement of conduction velocity were recorded by two pairs of small bipolar electrodes on the left and right atria, and were displayed on the other beam. The traces were photographed on film and measured subsequently with a projector. The stimulator was constructed by one of us and consisted of two channels, each comprising frequency, delay, duration and output stages, which could be locked together in various ways or could be operated independently. In the present experiments the output stages were employed as constant current devices over the range 20 μA to 25 mA, so that small changes of resistance in the tissue did not affect current strength. Stimulator A was used to provide the fundamental driving frequency and to trigger the oscilloscope sweep, and was connected to a pair of small platinum electrodes via an isolation transformer. Stimulator B was used to produce extra-systoles, flutter and fibrillation, and was directly coupled to a pair of silver-silver chloride disc electrodes of 2.8 mm diameter, placed above and below the left atrial tip. Drug concentrations have been expressed as w/v salt. Quinidine sulphate, papaverine sulphate and procaine hydrochloride were obtained from British Drug Houses, Dibenamine from Smith Kline and French, and procainamide from Squibb. The serpajmaline and ajmaline were provided by the kindness of Professor S. Siddiqui, of the Pakistan Council of Scientific and Industrial Research.

RESULTS

Experimental procedure. The actions of papaverine, quinidine sulphate, procaine hydrochloride, procainamide and Dibenamine were examined on a total of 41 pairs of rabbit atria and ajmaline and serpajmaline on three. The following measurements were made:

(1) The threshold for electrical excitation. Stimulator A was adjusted to a duration of 1.0 msec and a frequency of 180/min, which was in nearly every case faster than the spontaneous frequency. On a few occasions when the spontaneous frequency was faster than this, the stimulation frequency was set a little higher than the spontaneous frequency. Increasing current was then passed until the atria began to follow the stimulator. The thresholds were in the range 50 μA to 420 μA.

(2) Maximum driving frequency. The stimulus strength was raised to 2 mA and the frequency of stimulator A increased until the atria would no longer follow every stimulus.

(3) Threshold for extrasystoles. The preparation was again driven by A at 180/min. Stimulator B was set to a duration of 3 msec and a frequency of 600/min. The strength was increased until an extrasystole was observed.

(4) Threshold for flutter. The stimulus strength of B was further increased until a condition of flutter was induced, that is, until the atria broke away from both stimulators and beat at a fast regular frequency with co-ordinated contractions. The threshold for the induction of this effect proved to be sharp and reproducible.

(5) Threshold for fibrillation. The strength of the stimuli from stimulator B was further increased until flutter changed into fibrillation, that is, until the contractions became incoordinated. Here again a sharp and reproducible threshold was obtained. The length of time for which the fibrillation continued after cutting off stimulator B was very variable and could not be used as a test of any value.

(6) Conduction velocity. Action potentials from left and right atria were photographed while the atria were being driven at frequencies of 142 (if the spontaneous frequency was less than this), 180, 196, 244, 302, 367, 464/min, in so far as they would still follow. The change in conduction velocity after administration of the drug was then calculated separately at each frequency as a percentage of the control conduction velocity at that frequency. It was found that the percentage reduction in conduction velocity was always relatively a little greater at the higher frequencies, that is, the sensitivity of conduction velocity as a test of drug action was increased at the higher frequencies. For the purpose of plotting the log dose-response curve in Fig. 3, the percentage change was calculated as the mean change at all the frequencies measured.

(7) Contractions.

Experimental observations. Refractory period is affected by a number of factors, including the fundamental driving frequency and the strength of the conditioning stimulus (Dawes & Vane, 1956). Measuring the maximum frequency at which atria will follow a stimulus is not truly, therefore, a test of the effective refractory period, but Dawes (1946) showed that it was of great practical value in testing synthetic substitutes for quinidine. The results of this test are presented in Fig. 1, and show that quinidine is the most and procaine amide the least active substance. The parallelism of the dose-response curves is reasonable.

The measurements of the threshold for extrasystoles and for flutter put the drugs in the same order, but the dose-response curves were neither so straight nor so parallel as in the maximum frequency test. Consequently it has not been considered

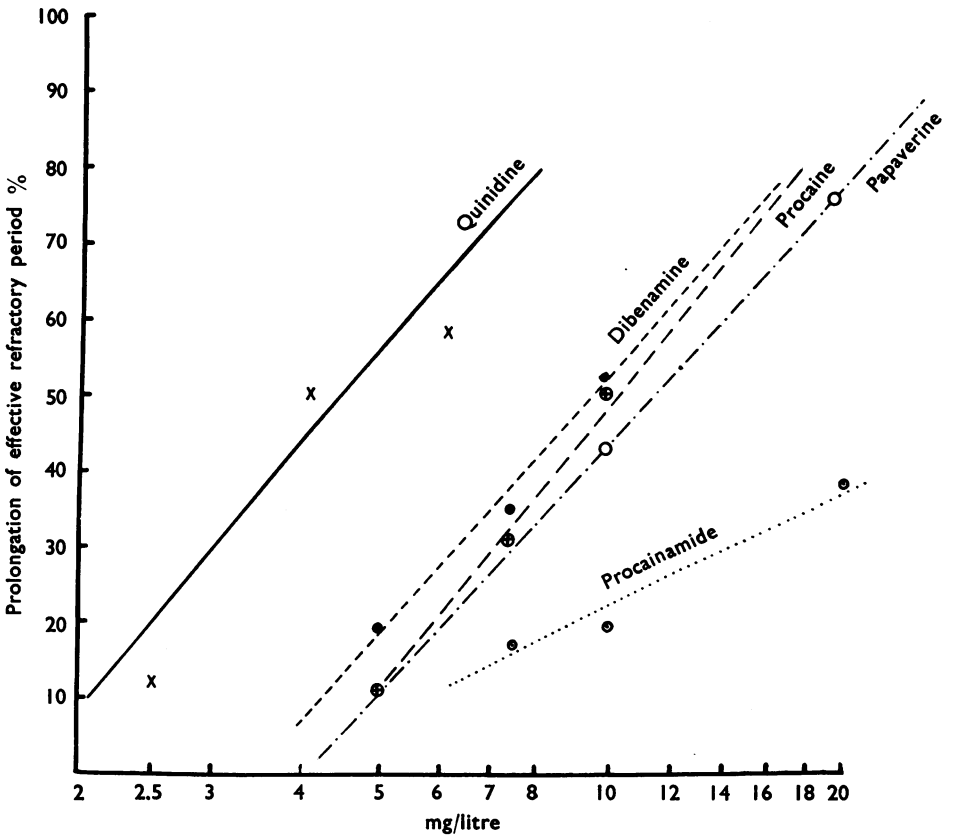

Fig. 1. Prolongation of effective refractory period. In this and subsequent figures the ordinates show % change, the abscissae drug concentration in mg/l. on a log scale. Crosses: quinidine. Dots: Dibenamine. Open circles: papaverine. Crossed circles: procaine. Dotted circles: procainamide.

worth plotting the results, as these two tests were obviously inferior. Measurements of the fibrillation threshold, on the other hand, gave results very similar to measurements of increase in effective refractory period in the maximum frequency test, and have been plotted in Fig. 2.

Conduction velocity was reduced by all the drugs, and, since the measurement of the distance between photographed action potentials is easy, accurate and entirely objective, it was hoped that conduction velocity measurements might afford a useful antifibrillatory test. The decreases in conduction velocity were relatively greater at the higher driving frequencies, which improved the discrimination. The results have been plotted in Fig. 3. It is at once apparent that the dose-response curves for Dibenamine, procaine and papaverine have quite different slopes from those for quinidine and procainamide. The implication is that, although all drugs with antifibrillatory action depress conduction velocity, the factors controlling conduction velocity are complex and are not necessarily affected to the same extent as the factors governing susceptibility to fibrillation. For this reason measurements of

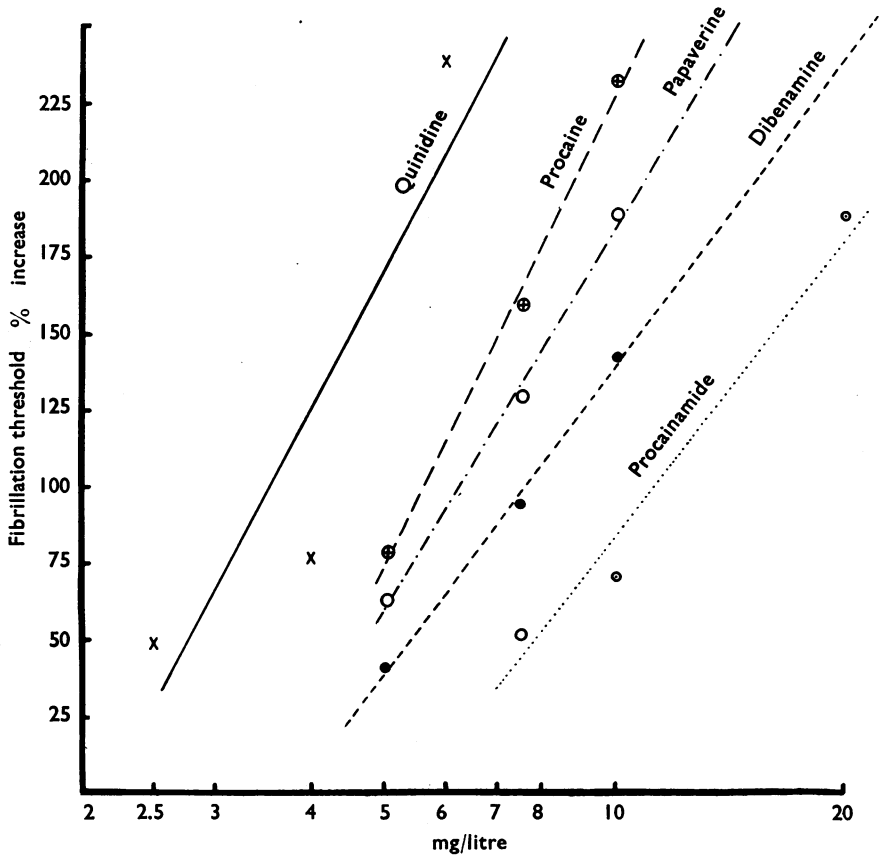

Fig. 2. Fibrillation threshold.

conduction velocity cannot be regarded as a satisfactory test for antifibrillatory activity. Finally, measurements of contraction also indicated a difference in slope in the dose-response curves for Dibenamine and procaine as compared with quinidine and procainamide (Fig. 4). Papaverine increased the contractions.

The least departures from parallelism in all the tests were between the results obtained with quinidine and procainamide, and it is therefore probable that these two drugs act in the same way. On fibrillation threshold procainamide had 0.32 the activity of quinidine and on contractions 0.29 the activity, but the difference between these two ratios was not statistically significant. These tests, therefore, show no therapeutic advantage of procainamide over quinidine. In Table 1 the mean doses of each drug required to produce a 100% increase in fibrillation threshold have been given in the first column. The second column indicates the mean changes in the size of contractions produced by these drug concentrations, and the third and fourth show changes in effective refractory period and conduction velocity respectively.

The fact that papaverine increased contractions although it had an antifibrillatory action, and that Dibenamine caused a relatively greater depression of contractions

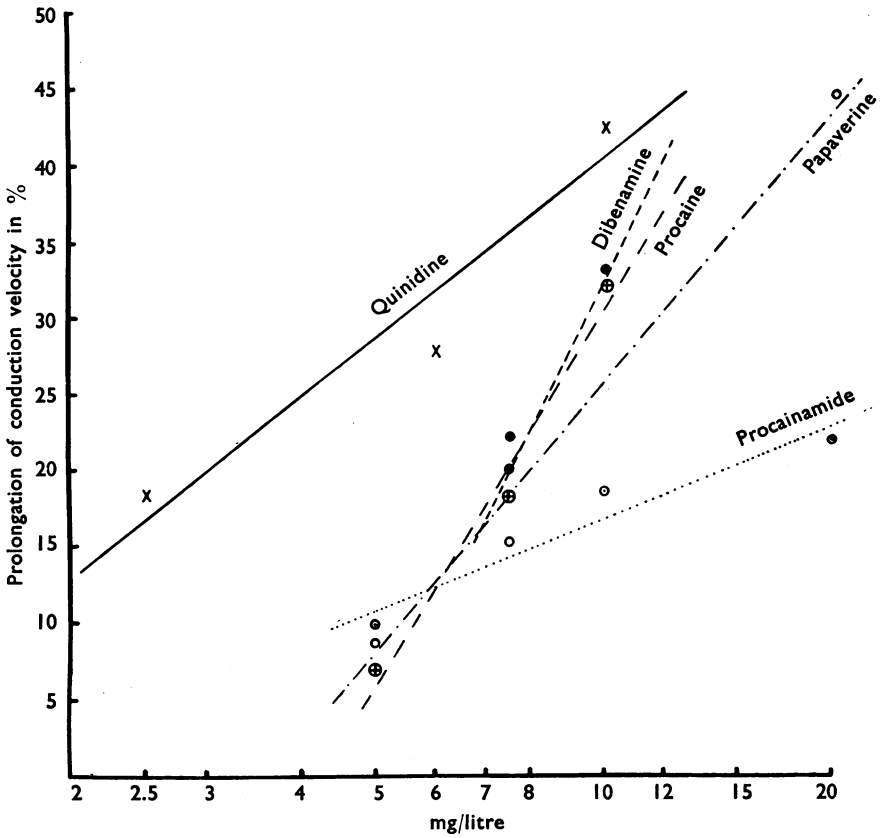

Fig. 3. Conduction velocity.

than the other drugs in relation to its antifibrillatory activity, indicated that the effects on susceptibility to fibrillation and contraction were at least separable, and gave some grounds for believing in the possibility of finding a more specific antifibrillatory compound. Serpajmaline has already been shown to have an antifibrillatory action (Child, Davis, Sharpe, Tomich & Deininger, 1959; Deininger, 1959). Insufficient experiments have been done so far to establish whether the mode of action is similar to that of the other drugs, but we have confirmed that serpajmaline contains a substance with an antifibrillatory action at least as powerful as that of quinidine, and with no more, and probably rather less, depressant action on contractions.

DISCUSSION

The object of the present work was twofold: first, to choose the most satisfactory test for antifibrillatory activity out of several that have been described; secondly, to put several drugs of different chemical structure in an order of antifibrillatory activity as a preliminary to an investigation of their mode of action. A quantitative comparison of quinidine, procaine, papaverine, Dibenamine and procainamide was undertaken, and a few experiments were also done with ajmaline and serpajmaline. Measurements of the maximum frequency at which atria would

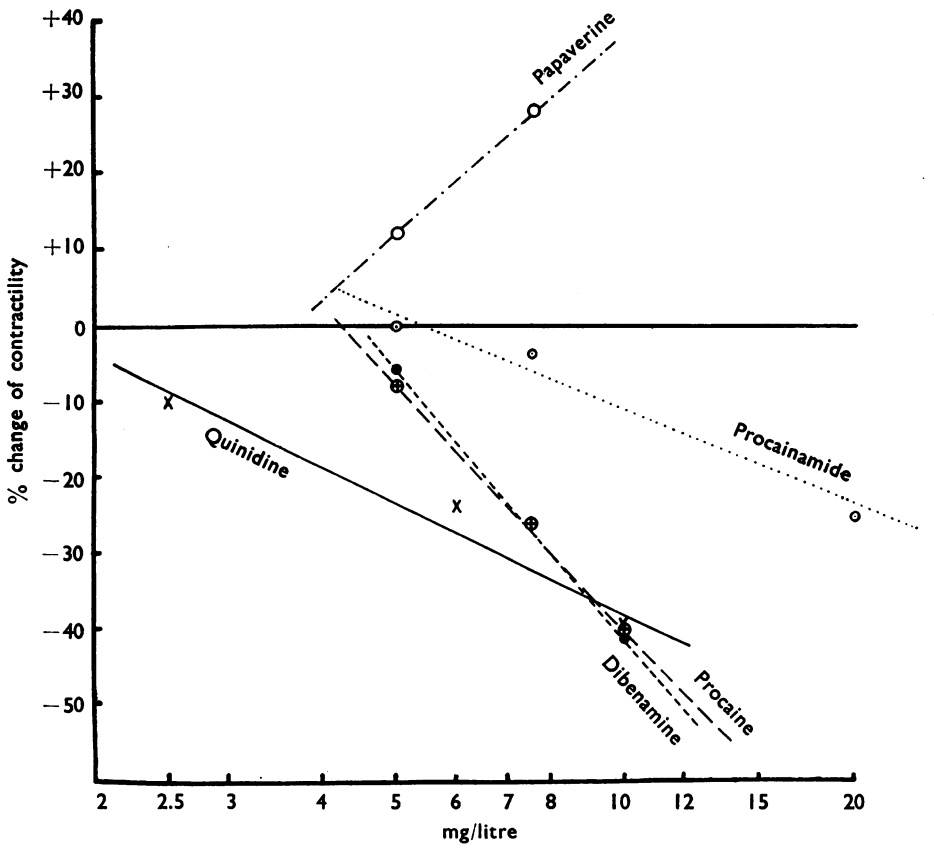

Fig. 4. Contractions.

follow a driving stimulus gave very similar results to measurements of the fibrillation threshold. Linearity of log dose-response and parallelism was much less good in measurements of the threshold for extrasystoles and for the development of flutter, and these tests were regarded as unsuitable for estimating antifibrillatory activity.

For quinidine and procainamide the regressions relating response and log dose were similar in all the tests, and it has been concluded that these drugs act in the

TABLE 1

In column 1 is given the mean concentration required to produce an increase of 100% in the fibrillation threshold. Column 2 shows the change in the magnitude of contractions produced by the concentrations in column 1, and columns 3 and 4 indicate the changes in refractory period and conduction velocity produced by these concentrations

	1 Conc. in mg/l.	2 Change in contraction %	3 Prolongation of refractory period %	4 Change in conduction velocity %
Quinidine	3·52	−14·8	+38	−22·5
Procaine	5·5	−11·5	+18	−11
Papaverine	6·2	+20·7	+21	−13
Dibenamine	7·51	−25·0	+38	−20·5
Procainamide	11·1	−12·5	+24·5	−18

same way, and that by the tests used neither had any advantage over the other. Procaine, papaverine and Dibenamine had very different regressions from quinidine and procainamide in measurements of conduction velocity and contraction amplitude. Dibenamine had a relatively greater depressant action on contractions than the other drugs, while papaverine increased contractions in association with an antifibrillatory action. This separation of effects on contraction from the reduction in susceptibility to fibrillation at least offers some hope of the possibility of obtaining a drug with a higher specificity of antifibrillatory action. Insufficient experiments have so far been done with ajmaline and serpajmaline, so that it is not known whether these substances act in the same way as the other drugs. But it was clear that serpajmaline contained a substance with an antifibrillatory action at least as powerful as that of quinidine, without any greater depressant action on contractions.

Measurements of fibrillation threshold and the maximum frequency at which atria would follow a stimulus gave very similar quantitative results, and, since the latter is an extremely easy test to perform, it is felt that the present work vindicates its use as a screening standard for antifibrillatory action (Dawes, 1946).

A possible explanation for the prolongation of the effective refractory period could be a prolongation of the action potential, but the available evidence is that clinically relevant concentrations of quinidine have no effect or only a trivial effect on the duration of the action potential (Wedd, Blair & Gosselin, 1942 ; Vaughan Williams, 1958b). Further, a prolongation of the action potential would offer no explanation for the other predominant effect of quinidine, a rise in threshold to electrical stimulation. More significance was, therefore, attached to the finding that quinidine, even in concentrations lower than those achieved in the blood of treated patients, caused a great slowing of the rate of the rise of the action potential in the absence of any fall in the resting potential (Vaughan Williams, 1958b). It was suggested that both the prolonged effective refractory period and the raised threshold could be accounted for by an interference by quinidine with the mechanism by which depolarizing ions were carried across the membrane. It has now been confirmed (Szekeres & Vaughan Williams, 1961) that all the drugs studied here also have in common with quinidine the property of greatly reducing the rate of rise of the action potential without affecting the resting potential or prolonging the time taken to repolarize to 50% of full repolarization. High concentrations prolonged the time for full repolarization by a few milliseconds, but this was quite inadequate to account for the large prolongation of the effective refractory period.

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