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A Cardiac Antiarrhythmic Screening Test Using the Isolated Ischaemic Perfused Rat Heart Preparation

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Abstract—Ventricular fibrillation was induced by myocardial ischaemia and reperfusion in the isolated perfused rat heart. The dose-response effectiveness for the prototype antiarrhythmic drugs, propranolol, quinidine and lidocaine in converting the induced ventricular fibrillation to sinus rhythm was determined. The test is easy to perform and does not require skillful surgical procedure and long time for observation. In addition, no arrhythmogenic drugs are used and the amount of substance needed for the test is very small. It is suggested that this simple test be used as a cardiac antiarrhythmic screening test for antiarrhythmic drugs.

Introduction

Cardiac arrhythmias are probably one of the major problems encountered in heart diseases. The search for experimental models for the evaluation of antiarrhythmic drugs has continued to be of interest to both experimental and clinical cardiologists. As yet, there is quite a number of methods available for the evaluation of antiarrhythmic property of drugs. According to Szekeres and Papp (1975), they can be classified into 4 categories, namely: 1, electrical stimulation of the heart; 2, mechanical or thermal production of local blocks and ectopic foci; 3, administration of drugs or chemicals into the animal intravenously or topically to the heart; 4, stimulation of the central nervous system. However, these techniques require skillful surgical operation or tedious procedures or long observation periods to induce a sufficiently high incidence of arrhythmias for the study.

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It has been shown that myocardial ischaemia and reperfusion lead to ventricular tachycardia (VT) and ventricular fibrillation (VF), both in the *in vitro* (Penny *et al.*, 1983) and *in vivo* preparations (Scheridan *et al.*, 1980). In the isolated perfused rat heart, we were also able to induce VF (or occasionally VT) easily by myocardial ischaemia and reperfusion (Zhan *et al.*, 1985). Since the method is simple, easy to perform and not time-consuming, we search for the possibility of using this ischaemia reperfusion-induced arrhythmias as a model for testing antiarrhythmic agents, using three established antiarrhythmic drugs (propranolol, quinidine and lidocaine) to evaluate it. In this report we describe the methodology in detail and the antiarrhythmic effects of the above mentioned drugs.

Methods

Perfusion of the isolated heart

Female Sprague-Dawley rats of 210–230 g were killed by decapitation. The heart was rapidly excised and mounted for perfusion by the Langendorff technique within 1 min. The perfusion fluid was Krebs-Ringer (pH = 7.4) containing (in mmol l^{-1}) NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.1, $NaHCO_3$ 24, $CaCl_2$ 2.5 and glucose 10, warmed by a water bath at a constant temperature of 37° C and equilibrated with 95 % O_2: 5 % CO_2 mixture throughout the experiment. A water jacket was also used to keep the temperature of the heart at 32° C during perfusion and 31° C during ischaemia. Preliminary study in our laboratory showed that a drop of temperature by 1° C did not change the electrocardiogram at all. The heart was perfused retrogradely at a constant perfusing pressure of about 100 mmHg with a constant flow rate of 6–8 ml per minute. Electrocardiograms were recorded by a Heart Monitor System Model No. 633 BM (Fukuda, Japan) with a positive electrode hooked at the apex of the heart, a negative electrode at the atrium and a ground electrode at the pericardial tissues. A microsyringe (Hamilton) was positioned via the aortic cannula for injection of drugs.

Induction of ventricular fibrillation by myocardial ischaemia and reperfusion

Immediately after the heart had been mounted, perfusion was stopped for 5, 10, 20 and 30 min, respectively followed by reperfusion. In this procedure there was no perfusion before and during ischaemia and the hearts would exhibit VF or occasionally VT 2–15 min after reperfusion. The optimal time of ischaemia needed for the highest percentage of the hearts exhibiting VF was then determined (Fig. 1) and this time (10 min) was used in the study of the antiarrhythmic property of drugs.

Drugs and assessment of antiarrhythmic activities

Propranolol from Imperial Chemical Industries (ICI), quinidine and lidocaine from Sigma, were dissolved in Krebs-Ringer. A fixed volume of 20 μl of the drugs or Ringer solution was injected into the preparation in one minute. The drug was administered after the appearance of VF induced by myocardial ischaemia and reperfusion. The doses in nmole/heart used in this study were as follows: 98, 196, 392, 784 for propranolol; 256, 512, 1024, 2048 for lidocaine and 185, 370, 740, 1480 for quinidine.

The antiarrhythmic activity was presented as cardiac antiarrhythmic protection (CAP) used by Baker and Erker (1980). It was considered to have CAP if VF was converted to sinus rhythm after administration of drug. In this study both the time course changes of CAP and relationship between doses and maximum CAP for each dose were determined. Regression lines showing dose and response relationship were determined by least square fit analysis. Each regression point represented data from 10 hearts.

Results

Figure 1 shows the percentage of hearts exhibiting VF with different times of myocardial ischaemia. Myocardial ischaemia for a period of

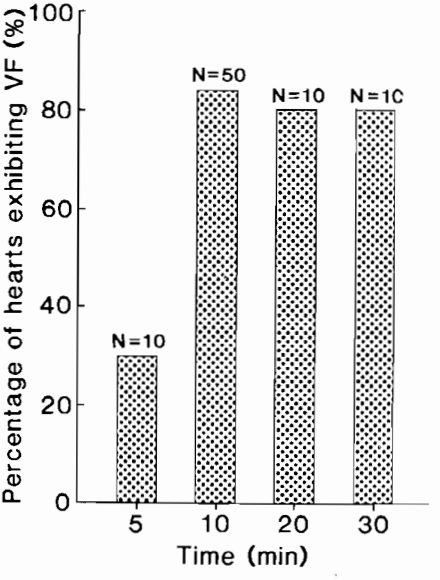

FIG. 1

Incidence of ventricular fibrillation following myocardial ischaemia and reperfusion in the isolated rat heart.

10 min resulted in the highest percentage of the hearts exhibiting VF during the subsequent reperfusion period, which was 84 %. We therefore used an ischaemic period of 10 min for the induction of VF in this study.

Figure 2 shows the time course changes in percentage of hearts recovered from VF after administration of the antiarrhythmic drugs propranolol, quinidine and lidocaine at the highest dose used in this study. The conversion from VF to sinus rhythm increased with time after administration of any of the three drugs up to 25 min. There was no more change in cardiac rhythm after 25 min, i.e. if the heart did not recover from VF before 25 min, it never recovered.

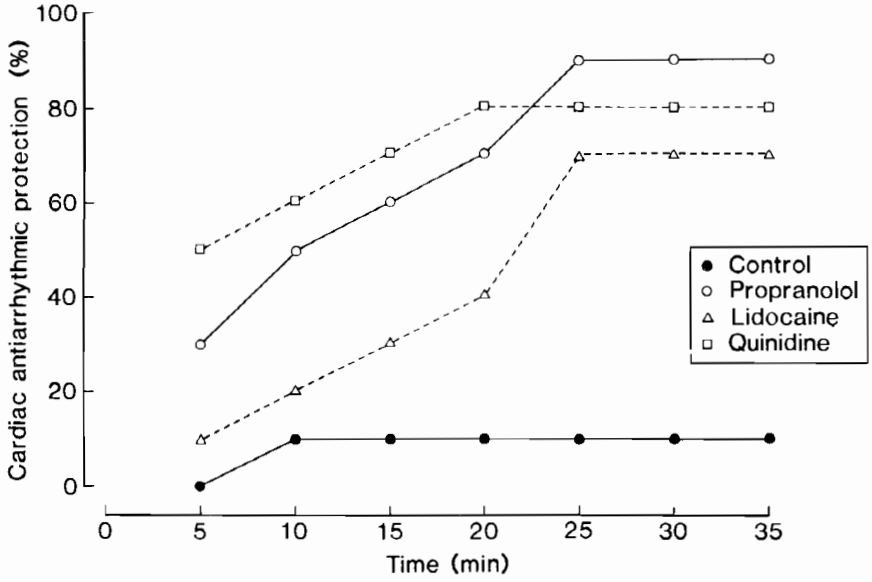

FIG. 2

Time course changes of conversion of ventricular fibrillation to sinus rhythm after administration of antiarrhythmic drugs in the isolated rat heart. The doses (nmole/heart) were 784, 2048 and 1480 for propranolol, lidocaine and quinidine, respectively. Numbers of hearts for each group were 10.

The maximum CAPs of the three drugs at 4 doses are shown in Fig. 3. All the drugs produced dose-dependent CAP as expected. The CAP_{50}s with 95 % confidence limit of propranolol, quinidine and lidocaine were 177.5 (156.8–201.0), 349.3 (325.0–375.4) and 509.6 (502.9–516.3) nmole/heart, respectively.

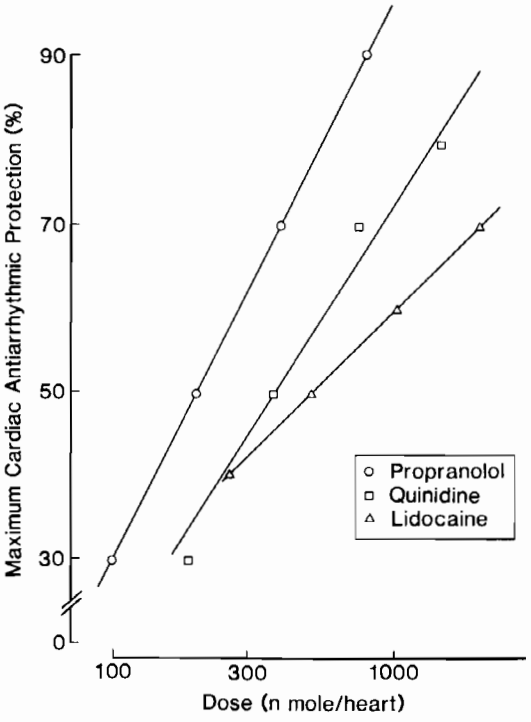

FIG. 3

The dose-maximum cardiac antiarrhythmic protection relationship for propranolol ($Y = 66.67\ \log X - 103$, $r = 1.00$), lidocaine ($Y = 33.00\ \log X - 39.30$, $r = 0.99$) and quinidine ($Y = 56.67\ \log X - 96.63$, $r = 0.99$). Each point represents data from 10 hearts.

Discussion

In this study, we showed that myocardial ischaemia followed by reperfusion induced a high incidence of VF in the isolated rat heart. 84 % of the hearts that had received this "ischaemic" treatment for 10 min exhibited VF during the reperfusion period (Fig. 1). The procedure did not require special surgical skill. It took only 2–15 min for VF to occur. In addition, no drug was used to induce cardiac arrhythmias. A very small amount of substance was needed for the test.

The antiarrhythmic activity of the drugs was assessed by their ability to convert VF to sinus rhythm. All three drugs possess this ability. The result is compatible with the well-established antiarrhythmic properties of these drugs and the results from the screening tests in other preparations (Wegria and Nickerson, 1943; Somani and Lum, 1965; Nwangwu *et al.*, 1977; Baker and Erker, 1980; Dai, 1982). The effectiveness of the antiarrhythmic activity was reflected by the dose-response relation curves.

Using this test we found that the relative antiarrhythmic potency of propranolol, quinidine and lidocaine is 1:0.51:0.35 from CAP_{50} values. This is very close to the relative potency of 1:0.67:0.36 for these drugs calculated from the results obtained from the chloroform hypoxia screening test by Baker and Erker (1980). It is also in agreement with the finding of Dai (1982), who found that quinidine was more effective than lidocaine in preventing ventricular arrhythmias in guinea-pig isolated heart preparation.

In conclusion we think that this technique is a good cardiac antiarrhythmic screening test because 1) it is simple; 2) it does not require skillful surgical technique; 3) it is not time-consuming; 4) it does not use arrhythmogenic drug for induction of cardiac arrhythmia; 5) the amount of substance needed for the test is very small.

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