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# DEVELOPMENT OF A SEVERE MODEL OF EARLY CORONARY ARTERY LIGATION-INDUCED DYSRHYTHMIAS IN THE ANAESTHETIZED RAT

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**1** The potential use of catecholamines to increase the severity of dysrhythmias evoked by coronary artery ligation in the anaesthetized rat was investigated. Drugs were given intravenously prior to ligation.

**2** Pressor doses of adrenaline (5 μg/kg) noradrenaline (1 μg/kg) phenylephrine (5–10 μg/kg), and angiotensin (0.25 μg/kg) conferred protection against the development of dysrhythmias.

**3** Atropine (1 mg/kg) increased mortality from ventricular fibrilloflutter (VF) and abolished the protective effects of phenylephrine (10 μg/kg).

**4** Administration of isoprenaline (10 μg/kg) significantly increased the incidence of and the mortality from VF.

**5** The order of antidysrhythmic drug potency of Org 6001 (1–10 mg/kg), disopyramide (2–10 mg/kg) and practolol (2–10 mg/kg) was similar in both the standard (without isoprenaline) and modified (with isoprenaline) models.

**6** Use of the modified method for antidysrhythmic screening purposes allows demonstration of statistically meaningful results with the use of relatively few animals.

**7** Comparison of the pattern of VF in the rat heart induced by various means suggests that the diagnosis of ventricular fibrillation can be made with more confidence in the modified method compared to the standard method.

## Introduction

Coronary artery ligation in the rat has, in recent years, been described as a method to produce dysrhythmias (Selye, Bajusz, Grasso & Mendell, 1960) and to assess potential antidysrhythmic activity (Au, Collins, Harvie & Walker, 1979; Clark, Foreman, Kane, McDonald & Parratt, 1980). Assessment of antidysrhythmic activity has been based both on reduction in the expected number of ventricular ectopic beats and in the incidence of ventricular fibrillation. An incidence of ventricular fibrillation (VF) of 28 to 70%, which in the anaesthetized rat may spontaneously revert to normal sinus rhythm (NSR) has been reported (Au *et al.*, 1979; Clark *et al.*, 1980; Kane & Winslow, 1980). Diagnosis of dysrhythmia types in the rat is made difficult because of high control heart rates (usually greater than 400 beats/min) combined with the effects of ligation to produce, in many instances, complete fusion of the QRS and T waves. Thus, it is often difficult to distinguish between ventricular flutter and ventricular fibrillation at least in those animals whose rhythm reverts to NSR; arterial blood pressure often has to be used as a guide. Unfortunately, the literature contains very few pictorial examples of VF in the rat following ligation and therefore it is difficult to compare ECG interpretations made by different authors. We have been concerned for some time whether the incidence of true VF is in fact as high as the above quoted figures and for this reason we have sought to produce a more severe model of coronary artery ligation in which VF may be diagnosed with more confidence. Since evidence is accumulating to suggest that increased sympathetic activity is a factor in the genesis of lethal arrhythmias (Opie & Lubbe, 1979), we have studied the effects of catecholamines on the severity of dysrhythmias evoked by acute coronary artery ligation. The results of this study showed that isoprenaline greatly exacerbated dysrhythmias caused by ligation. The effects of the β-adrenoceptor blocking agent, practolol, and of the antidysrhythmic drugs, Org 6001 and disopyramide, on the development of these dysrhythmias were therefore determined to assess the potential use of the isoprenaline-sensitized rats for antidysrhythmic screening purposes. Evidence is also presented to suggest that diagnosis of ventricular fibrillation may be inaccurate in animals that revert to NSR.

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## Methods

The method used to induce dysrhythmias in the anaesthetized rat by coronary artery ligation has been described in full by Clark *et al.* (1980). Male Wistar rats (320–480 g) were anaesthetized with pentobarbitone sodium (60 mg/kg given i.p.) and artificially ventilated with room air (stroke volume, 4 ml; 48 strokes/min). Arterial blood pressure (BP) was recorded from the right carotid artery and the electrocardiogram (ECG) (Lead II) recorded from subcutaneous steel needle electrodes. BP and the ECG were displayed on a Minograph 82 ink jet recorder (Elma-Schonander). A left thoracotomy was performed, the heart exteriorized, and a 6/0 silk suture placed under the main left coronary artery as described by Selye *et al.* (1960).

The heart was repositioned in the thoracic cavity and a stabilization period of 15 min allowed. Drugs were given via the left femoral vein, 1 min before tightening the ligature. The number of premature ventricular systoles (PVS) was counted during the 0–30 min post ligation period. The incidence of ventricular fibrillo flutter (VF) was also determined.

Drugs used were (±)-isoprenaline hydrochloride (Riker), (±)-adrenaline hydrochloride (Riker), (−)-noradrenaline base (Sigma), (±)-phenylephrine hydrochloride (Boots Co. Ltd), $Val_5$-Hypertensin II-Asp-β-amide (Angiotensin) (Ciba), atropine sulphate (Sigma), Org 6001 hydrochloride (Organon International), disopyramide phosphate (Searle), and practolol hydrochloride (Imperial Chemical Industries).

A Chi-square test was used to detect significant differences in the incidence of VF between control and drug-treated animals. For differences between means, Student's *t* test was used to determine significance.

**Table 1** The effects of catecholamines and angiotensin injected intravenously 1 min before ligation on the development of dysrhythmias in the anaesthetized rat

| *Treatment* (μg/kg) | *n* | *PVS* (0–30 min) | *% incidence of VF* | *% mortality from VF* |
|---|---|---|---|---|
| Saline (1 ml/kg) | 27 | 1306 ± 174 | 52 | 7 |
| Isoprenaline (5) | 6 | 2178 ± 248** | 67 | 17 |
| Isoprenaline (10) | 10 | 3008 ± 917 | 90* | 50** |
| Adrenaline (1) | 7 | 909 ± 487(6)<br>4677(1) | 29 | 0 |
| Adrenaline (5) | 7 | 304 ± 151*** | 0* | 0 |
| Noradrenaline (1) | 8 | 843 ± 258 | 0* | 0 |
| Phenylephrine (5) | 6 | 1321 ± 300 | 17 | 0 |
| Phenylephrine (10) | 13 | 757 ± 228* | 23 } * | 15 |
| Atropine (1000) | 9 | 735 ± 298 | 44 | 44** |
| Atropine (1000) + phenylephrine (10) | 8 | 956 ± 574 | 50 | 38* |
| Angiotensin (0.25) | 7 | 252 ± 94*** | 29 | 0 |

Atropine was given 15 min before ligation.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (significantly different from saline-treated group).
*n* = number of animals. PVS and VF denote premature ventricular systoles and ventricular fibrillo flutter respectively.

## Results

*Effects of catecholamines, angiotensin and atropine on the development of dysrhythmias*

Coronary artery ligation in 27 control animals resulted in a mean ventricular ectopic count of 1306 ± 174 beats and a 52% incidence of VF during the 30 min postligation period. In all animals no dysrhythmias were observed after this time. Table 1 summarizes the effects of isoprenaline, adrenaline, noradrenaline and phenylephrine on the number of PVS and the incidence of VF. Both adrenaline (5 μg/kg) and noradrenaline (1 μg/kg) prevented the development of VF following coronary artery ligation and the combined incidence of VF in the phenylephrine groups was also significantly smaller than that observed in the controls. A significant reduction in the number of PVS was also seen in the 5 μg/kg adrenaline and 10 μg/kg phenylephrine groups.

The protective effects of adrenaline, noradrenaline and phenylephrine against ligation-induced dysrhythmias were accompanied by hypertension (Table 2). Mean arterial blood pressures rose significantly by 52, 55 and 62% in response to adrenaline (5 μg/kg), noradrenaline (1 μg/kg) and phenylephrine (10 μg/kg) respectively whereas heart rates remained essentially unchanged (Table 3). Twenty minutes after ligation, arterial blood pressures in the control, adrenaline (1 μg/kg) and noradrenaline (1 μg/kg) groups were modestly reduced below the values recorded prior to saline or drug administration. The differences reached significance in the control and noradrenaline (1 μg/kg) groups. Arterial blood pressure in the 5 μg/kg adrenaline group was unchanged at this time whilst small but insignificant increases in arterial pressure were recorded in the animals given phenylephrine (5 or 10 μg/kg).

In all groups, with the exception of the 5 μg/kg phenylephrine group, cardiac rates recorded 20 min postligation were lower than those recorded at the start of the experiments. The differences in rates were significant only for the noradrenaline (1 μg/kg) group.

Angiotensin (0.25 μg/kg) also significantly reduced the number of PVS evoked by coronary artery ligation and decreased, but not significantly, the incidence of VF (Table 1). This agent increased mean arterial pressure by 42% (Table 2) whilst heart rate remained unchanged (Table 3). Twenty minutes after ligation, blood pressure was similar to the predrug value and heart rate significantly reduced.

Atropine (1 mg/kg given 15 min before ligation) significantly increased mortality from VF and completely prevented the protective effects of phenylephrine (10 μg/kg) against ligation-induced dysrhythmias (Table 1). In atropine-treated animals that did not receive phenylephrine, blood pressures and heart rates were similar when recorded pre and 20 min post ligation (Tables 2 and 3). Animals that received both atropine and phenylephrine showed a

**Table 2** Arterial blood pressure recorded from rats before and after treatment and before and 20 min after ligation

| | | *Systolic/Diastolic blood pressure* (mmHg) | | | |
|---|---|---|---|---|---|
| *Treatment* | *Dose* (μg/kg) | *Predrug* | *Maximum* | *Preligation* | *20 min post ligation* |
| Saline | 1 ml/kg | 120 ± 4/ 94 ± 5 | | 120 ± 4/94 ± 5 | * 114 ± 6/ 87 ± 8 |
| Isoprenaline | 5 | 156 ± 7/106 ± 8 | ** 105 ± 10/ *** 43 ± 2 | ** 105 ± 10/ *** 43 ± 2 | * 126 ± 9/ 92 ± 12 |
| Isoprenaline | 10 | 122 ± 11/ 90 ± 10 | ** 76 ± 5/ *** 40 ± 2 | ** 80 ± 5/ ** 45 ± 4 | 107 ± 17/ 83 ± 21 |
| Adrenaline | 1 | 127 ± 13/ 96 ± 14 | 150 ± 5/108 ± 7 | 141 ± 5/ 99 ± 7 | 116 ± 8/ 89 ± 9 |
| Adrenaline | 5 | 148 ± 7/102 ± 10 | *** 201 ± 8/*** 167 ± 9 | ** 189 ± 6/** 144 ± 7 | 144 ± 5/103 ± 5 |
| Noradrenaline | 1 | 118 ± 6/ 85 ± 5 | *** 178 ± 7/*** 135 ± 5 | *** 169 ± 8/** 129 ± 8 | * 101 ± 7/ ** 63 ± 6 |
| Phenylephrine | 5 | 139 ± 11/107 ± 12 | * 184 ± 9/* 150 ± 8 | * 176 ± 6/* 145 ± 7 | 149 ± 14/118 ± 17 |
| Phenylephrine | 10 | 121 ± 6/ 95 ± 6 | **** 193 ± 6/**** 151 ± 3 | **** 175 ± 7/**** 141 ± 3 | 125 ± 8/100 ± 9 |
| Angiotensin | 0.25 | 112 ± 7/ 87 ± 7 | ** 159 ± 7/*** 130 ± 7 | ** 144 ± 7/** 119 ± 6 | 117 ± 5/ 82 ± 9 |
| Atropine | 1000 | 110 ± 9/ 81 ± 14 | | 110 ± 9/ 81 ± 14 | 112 ± 11/ 82 ± 14 |
| Atropine + Phenylephrine | 1000 + 10 | 116 ± 7/ 95 ± 12 | ** 159 ± 7/* 125 ± 8 | ** 157 ± 7/* 124 ± 8 | 95 ± 7/ 75 ± 12 |

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; **** $P<0.001$ (significantly different from pretreatment values).
The results are the mean ± s.e.mean of from 5 to 27 observations.

**Table 3** Heart rates recorded from rats before and after treatment and before and 20 min after ligation

| Treatment | Dose (μg/kg) | *Heart rate* (beats/min) Predrug | Maximum | Preligation | 20 min post ligation |
|---|---|---|---|---|---|
| Saline | 1 ml/kg | 418 ± 12 | | 418 ± 12 | 391 ± 12 |
| Isoprenaline | 5 | 440 ± 3 | 464 ± 7 * | 464 ± 7 * | 396 ± 11 * |
| Isoprenaline | 10 | 435 ± 12 | 478 ± 13 * | 485 ± 9 ** | 402 ± 23 |
| Adrenaline | 1 | 422 ± 21 | 420 ± 22 | 422 ± 23 | 367 ± 15 |
| Adrenaline | 5 | 420 ± 14 | 420 ± 15 | 420 ± 15 | 385 ± 10 |
| Noradrenaline | 1 | 404 ± 17 | 415 ± 15 | 410 ± 16 | 346 ± 19 * |
| Phenylephrine | 5 | 423 ± 17 | 417 ± 13 | 417 ± 13 | 424 ± 12 |
| Phenylephrine | 10 | 424 ± 16 | 404 ± 14 | 424 ± 13 | 391 ± 11 |
| Angiotensin | 0.25 | 430 ± 9 | 438 ± 6 | 430 ± 13 | 388 ± 16 * |
| Atropine | 1000 | 413 ± 10 | | 385 ± 9 | 396 ± 11 |
| Atropine + Phenylephrine | 1000 10 | 456 ± 34 | 457 ± 31 | 457 ± 31 | 444 ± 26 |

* $P < 0.05$; ** $P < 0.01$ (significantly different from pretreated values). The results are the mean ± s.e.mean of from 5 to 27 observations.

modest but statistically insignificant fall in pressure 20 min after ligation and heart rate was little changed.

Isoprenaline (10 μg/kg) significantly increased the incidence of and the mortality from VF (Table 1). Both doses of isoprenaline (5 and 10 μg/kg) significantly reduced blood pressure and modestly increased heart rate (Tables 2 and 3). Twenty minutes after ligation, blood pressures and heart rates were reduced to an extent similar to that observed in the control animals.

*Effects of antidysrhythmic agents on dysrhythmias evoked by coronary artery ligation in animals pretreated with isoprenaline (10 μg/kg)*

Coronary artery ligation in 33 animals given 10 μg/kg isoprenaline 1 min before ligation resulted in a significantly greater number of PVS and a significant increase in the incidence of VF and in mortality compared to control animals. Table 4 compares the effects of Org 6001, practolol and disopyramide on the development of ligation-evoked dysrhythmias in animals with (modified method) and without (standard method) isoprenaline. All three drugs exhibited antidysrhythmic activity in both models. It is also apparent from the results that the levels of significance between control and drug treated groups are much higher in the animals given intravenous isoprenaline both with respect to the number of PVS and the incidence of VF. Thus, using the modified method, statistically meaningful results can be obtained for reduction in VF using relatively small numbers of animals. Significant decreases in mortality from VF were also detected in the disopyramide (5 and 10 mg/kg) and Org 6001 (5 mg/kg) groups.

Figure 1 compares log dose-response lines to the three drugs for the number of PVS and the incidence of VF in the standard and modified methods. The main point that this figure illustrates is that clear dose-related antidysrhythmic effects of the three drugs can be seen with the modified method. Approximate $ED_{50}$ values are listed in Table 5. In the modified method, the $ED_{50}$ values for PVS and VF were similar for each drug tested. The order of potency was Org 6001 > disopyramide > practolol. A similar order of potency was obtained in the standard method for reduction in the number of PVS. The slope of the log dose-response line to practolol was however significantly less ($P < 0.01$) than that obtained using the modified method whereas comparison of the slopes of the regression lines in either the Org 6001 or disopyramide groups were similar. Practolol and disopyramide were approximately equipotent in reducing the incidence of VF and Org 6001, again was the most potent.

*Diagnosis of ventricular fibrillation in the rat heart*

As stated in the introduction, we are not certain that true ventricular fibrillation occurs in response to coronary artery ligation in those animals that revert

**Table 4** Comparative antidysrhythmic effects in animals with or without isoprenaline

| | | *Without isoprenaline* | | | | *With isoprenaline* | | |
|---|---|---|---|---|---|---|---|---|
| *Treatment* | *Dose* (mg/kg) | n | *PVS* (0–30 min) | *% incidence of VF* | *% mortality from VF* | n | *PVS* (0–30 min) | *% incidence of VF* | *% mortality from VF* |
| Saline | 1 ml/kg | 27 | 1306 ± 174 | 52 | 7 | 33 | 2664 ± 463 † | 82 † | 39 † |
| Org 6001 | 1 | | | | | 8 | 1355 ± 482 * | 38 * | 13 |
| | 2 | 6 | 595 ± 213 * | 17 | 0 | 7 | 1143 ± 537 * | 14 ** | 0 * |
| | 5 | 8 | 681 ± 463 | 0 * | 0 | | | | |
| | 10 | 6 | 235 ± 63 ** | 0 | 0 | 6 | 15 ± 12 **** | 0 *** | 0 |
| Practolol | 2 | 7 | 954 ± 373 | 29 | 14 | 5 | 3078 ± 430 | 80 | 40 |
| | 5 | | | | | 7 | 1580 ± 524 | 43 | 43 |
| | 10 | 8 | 699 ± 299 * | 13 | 13 | 7 | 493 ± 374 *** | 29 * | 14 |
| Disopyramide | 2 | 6 | 928 ± 293 | 67 | 0 | 7 | 2187 ± 565 | 71 | 29 |
| | 5 | 8 | 857 ± 273 | 13 | 13 | 8 | 1007 ± 306 ** | 38 * | 0 * |
| | 10 | 6 | 217 ± 94 ** | 0 | 0 | 8 | 278 ± 246 *** | 0 *** | 0 * |

† Significantly different from control value in the absence of isoprenaline.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$, denote significant differences from corresponding saline-treated animals.
PVS and VF denote premature ventricular systoles and ventricular fibrillo flutter respectively.

spontaneously to NSR. Figure 2 shows a typical record of the pattern of dysrhythmias which we have designated as VF. One of the uncertain aspects as regards interpretation of this dysrhythmia as ventricular fibrillation is that the amplitude of the fibrillatory waves is often larger than that of the normal R wave and that arterial blood pressure during VF rarely falls below 20 mmHg and often shows small pressure waves. In other species the amplitude of the fibrillatory waves is invariably smaller than the amplitude of the R wave. Thus the dysrhythmia shown in Figure 2 may equally well be interpretated as multifocal ventricular extrasystoles, flutter or 'torsades de pointes' tachycardia. Figure 3a shows a typical record of ventricular fibrillation induced by coronary artery ligation followed by reperfusion in the isolated heart of the rat. In this instance, the amplitude of the fibrillatory waves is much smaller than that of the R wave and contractility has completely ceased. Figure 3b shows a record taken from a rat in which VF was induced electrically (510 μA current, 0.8 ms, 50 Hz). Again, the amplitude of the fibrillatory waves is, on the whole, smaller than the amplitude of the normal R wave and arterial blood pressure has fallen to 5 mmHg and shows no evidence of pressure waves.

**Table 5** Approximate antidysrhythmic $ED_{50}$ values (mg/kg)

| *Treatment* | *With isoprenaline* | | *Without isoprenaline* | |
|---|---|---|---|---|
| | *PVS* | *VF* | *PVS* | *VF* |
| Org 6001 | 1.1 | 0.9 | 3.0 | 1.2 |
| Practolol | 5.8 | 5.4 | 12.0 | 2.3 |
| Disopyramide | 3.9 | 4.6 | 6.2 | 3.9 |

PVS and VF denote premature venticular systoles and ventricular fibrillo flutter respectively.

In the isoprenaline-treated rats subjected to coronary artery ligation the 'fibrillatory' pattern in animals that reverted to NSR was similar to that recorded in control animals, whereas in those that did not revert to NSR the amplitude of the fibrillatory waves became progressively smaller and more closely resembled fibrillation recorded in other species (Figure 4). Cardiovascular collapse resulted in 40% of these animals.

## Discussion

The main aim of the present study was initially to find a model of coronary artery ligation-induced dysrhythmias which gave a sufficient incidence of VF for drug screening purposes. Injection of adrenaline, noradrenaline and phenylephrine, in doses sufficient to increase mean arterial blood pressure by approximately 50%, were found to antagonize the development of dysrhythmias evoked by coronary artery ligation. Since angiotensin also exerted a protective effect, it seems unlikely that α-adrenoceptor stimulation plays a direct role in the protective effects of these catecholamines. It is more likely that reflexly

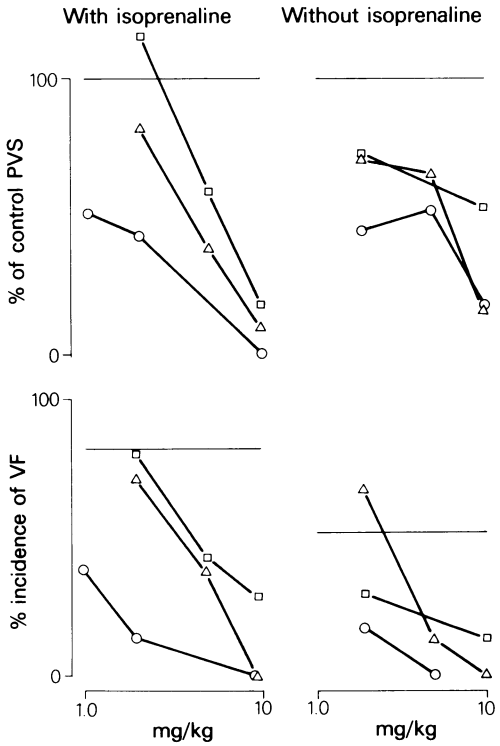


**Figure 1** Log dose-response lines to Org 6001 (○), practolol (□) and disopyramide (△) for reduction in the number of premature ventricular systoles (PVS) and in the incidence of ventricular fibrillation in animals subjected to coronary artery ligation with or without isoprenaline. The horizontal bars on each graph depict values obtained in the appropriate control groups.

induced increases in vagal tone produced by the rise in blood pressure account for the antidysrhythmic actions of these agents. Verrier, Calvert, Lown & Axelrod (1974) and Verrier & Lown (1978) have shown that blood pressure elevation by phenylephrine increases the ventricular fibrillation threshold (VFT) in the dog and have provided evidence to suggest that this protection is due to reflex decreases in sympathetic tone mediated by the vagus. Preliminary experiments in our own laboratories suggest that vagal stimulation during myocardial ischaemia in the rat protects against the development of dysrhythmias. In these experiments the mean number of PVS in 5 animals was only $765 \pm 238$ and VF was absent. The results of Verrier *et al.* (1974), and Verrier & Lown (1978) also suggest that vagal influences on susceptibility to fibrillation are indirect and depend on the level of pre-existing sympathetic tone. In the present experiments, a high sympathetic tone is reflected in the rapid resting heart rates and would be expected in open-chest animals anaesthetized with pentobarbitone. We were also able to show that atropine, although without effect on the expected number of PVS or the incidence of VF, significantly increased mortality from VF. The same dose of atropine also prevented the antidysrhythmic actions of phenylephrine, again suggesting that protection by phenylephrine is mediated via increased vagal tone. Similar exacerbation of ischaemia-induced dysrhythmias by atropine has also been demonstrated both experimentally and clinically (Webb, Adgey & Pantridge, 1972; Corr & Gillis, 1974). Although phenylephrine and angiotensin exerted antidysrhythmic actions, we were surprised to find that adrenaline and noradrenaline also conferred protection since the actions of these drugs are

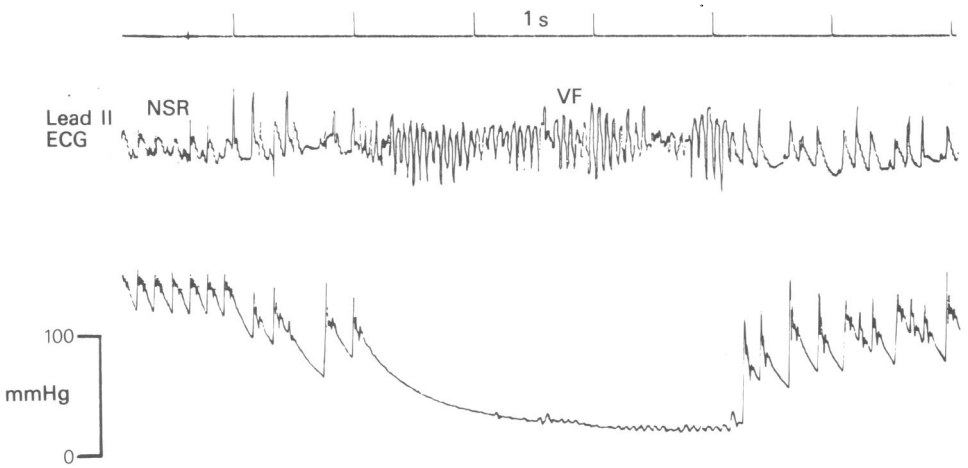


**Figure 2** An example of a dysrhythmia diagnosed as ventricular fibrilloflutter (VF) is an anaesthetized rat following coronary artery ligation. NSR is normal sinus rhythm.

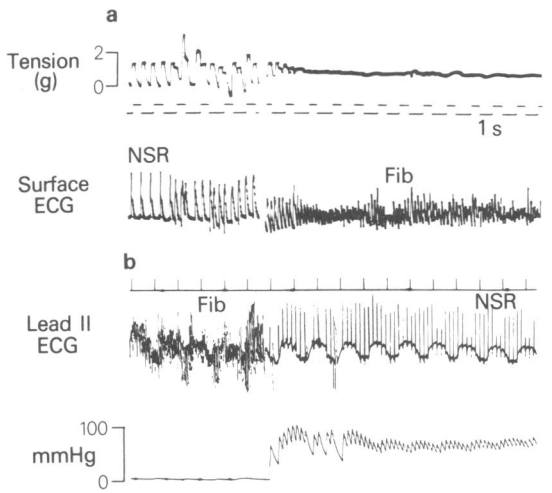


**Figure 3** (a) An example of ventricular fibrillation (Fib) induced by 20 min of coronary artery ligation followed by reperfusion in an isolated perfused heart of the rat. (b) Ventricular fibrillation induced in the rat heart *in vivo* by electrical stimulation of the left ventricle (510 μA; 50 Hz, 0.8 ms) NSR is normal sinus rhythm.

associated with aggravation of dysrhythmias during experimental myocardial infarction (Maling & Moran, 1957). Also, increased sympathetic activity early in myocardial infarction can be demonstrated in association with the occurrence of dysrhythmias (McDonald, Baker, Bray, McDonald & Restieaux, 1969; Vetter, Strange, Adams & Oliver, 1974; Pantridge, Adgey, Geddes & Webb, 1975). However, in view of the nature of the model we used, where a high sympathetic tone is already evident, it may be that pressor-mediated reflex increases in vagal activity are sufficient to overide any of the cardiac excitant effects of exogenous catecholamines. It is also possible that increased coronary perfusion pressure consequent upon raised diastolic pressure may also play a protective role.

In contrast to the effects of these catecholamines on the development of ligation-induced dysrhythmias, 10 μg/kg isoprenaline given 1 min before ligation markedly increased the number of PVS, the incidence of VF and the mortality from VF. The increased sensitivity to dysrhythmias may be due to both a reflexly-induced increase in sympathetic activity in response to hypotension or may be due to a direct effect of isoprenaline on cardiac conduction. Mean arterial blood pressure was reduced by 57% by this dose of isoprenaline and heart rate increased by 10%. It is also highly likely that the metabolic effects of isoprenaline on the myocardium contribute to its ability to exacerbate dysrhythmias. For instance, it has been shown that the incidence of ventricular dysrhythmias and fibrillation produced by coronary artery ligation correlate well with increases in cyclic adenosine 3′,5′-monophosphate (cyclic AMP) in both normal and ischaemic myocardium of anaesthetized cats (Corr, Witkowski & Sobel, 1978), dogs and baboons (Podzuweit, Dalby, Cherry & Opie, 1978). Lubbe, Bricknell, Podzuweit & Opie (1976) have also shown, using rat isolated hearts, that dibutyryl cyclic AMP lowers the ventricular fibrillation threshold and increases the width of the vulnerable period whilst Opie & Lubbe (1979) have demonstrated a correlation between increased cyclic AMP in response to adrenaline and lowering of VFT. Com-

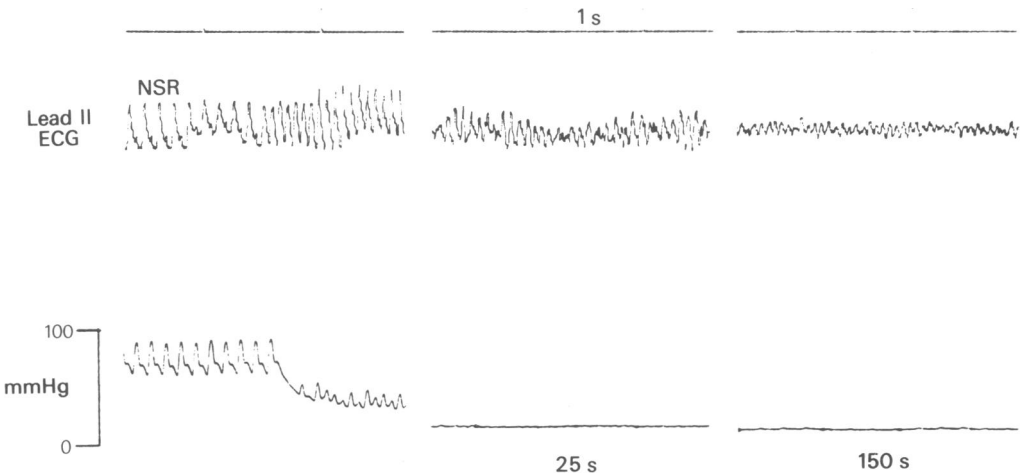


**Figure 4** Ventricular fibrilloflutter (or fibrillation) induced by coronary artery ligation in a rat given 10 μg/kg of isoprenaline 1 min before ligation. This animal did not revert to normal sinus rhythm (NSR). The numbers below the panels denote the time (s) after the onset of the dysrhythmia.

parison of the slopes of the log dose-response lines to practolol in the standard and modified methods in the present study also suggests that stimulation of myocardial $\beta_1$-adrenoceptors by isoprenaline is responsible for exacerbation of dysrhythmias since the slope obtained with the modified method was significantly steeper than that obtained by the standard method.

Using Vaughan Williams (1970) classification of antidysrhythmic drugs, the class I antiarrhythmic agents, Org 6001 (Salako, Vaughan Williams & Wittig, 1976) and disopyramide (Sekiya & Vaughan Williams, 1963), as well as the cardioselective $\beta_1$-adrenoceptor antagonist practolol were effective in antagonizing dysrhythmias in the modified method, in doses comparable to those required to exert an antidysrhythmic effect in the standard method. The order of potency was similar in both models. It is therefore felt that the modified method is valid for screening purposes. Advantages of this method are: (1) statistically meaningful results can be obtained using relatively smaller numbers of animals, (2) analysis of results is very much more rapid because only VF is considered as an endpoint and (3) approximate $ED_{50}$ values for reduction in VF can be more easily obtained since the control incidence of VF is substantially higher in the modified method.

We also feel that diagnosis of ventricular fibrillation can be made with more confidence in isoprenaline-treated animals since the pattern of VF certainly in animals failing to revert to NSR consisted of low amplitude irregular waves accompanied by arterial blood pressures of 15 mmHg or less. With the modified method, cardiovascular collapse was seen in 40% of the animals (compared to 7% in the standard method). Unfortunately, there are few illustrations in the literature of the dysrhythmic rat ECG so that at present spontaneously reverting 'fibrillation' remains ill-defined. Lubbe, Bricknell & Marzagao (1975) depict low amplitude electrically induced ventricular fibrillation in the isolated heart of the rat similar to the pattern we have recorded in perfused isolated heart (Figure 3a) in anaesthetized rats with electrically-induced fibrillation (Figure 3b) and in coronary-ligation rats treated with isoprenaline. In these instances fibrillation is accompanied by complete loss of contractility or arterial pressure records (0–15 mmHg) showing no oscillation. Measurement of cardiac output during spontaneously reverting VF may help to clarify the diagnosis as flutter or true fibrillation.

In conclusion, injection of 10 $\mu$g/kg isoprenaline, 1 min before ligation, results in exacerbation of the resulting dysrhythmias. The increased incidence of VF and the higher mortality allow demonstration of statistically meaningful antidysrhythmic actions of test drugs using relatively small numbers of animals. In addition we feel that diagnosis of ventricular fibrillation can be made with more confidence in this modified model.

The results of the present study also suggest that pressor agents confer protection against dysrhythmias evoked by coronary artery ligation in the rat, possibly by indirect suppression of sympathetic activity reflexly mediated through the vagus. This mechanism should be taken into account when testing new potential antidysrhythmic agents in this model.

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