Effects of antiarrhythmic drugs on ventricular fibrillation thresholds of normal and ischaemic myocardium in the anaesthetized rat

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1   The effects of agents which produce membrane stabilization (class I), β_1-adrenoceptor blockade (class II), prolongation of the cardiac action potential (class III) or inhibition of the slow inward current (class IV) were investigated for their ability to increase the ventricular fibrillation threshold (VFT) or to modify the fall in VFT consequent upon coronary artery ligation in the anaesthetized rat.

2   The class I agent, Org 6001, increased VFT of normal myocardium and in lower doses reduced the postligation fall in VFT.

3   The class II agent, metoprolol, failed to increase VFT of normal myocardium but reduced the postligation fall.

4   The class III agent, melperone, increased VFT of both normal and ischaemic myocardium whereas the class IV agent, nifedipine failed to influence VFT in either region.

5   Bepridil (class I and IV) was similar to Org 6001 and sotalol (class II and III) in that it increased VFT of normal myocardium and in lower doses reduced the postligation fall in VFT.

6   Measurement of VFT before and after coronary artery ligation in the rat constitutes a rapid and reproducible screen to detect antifibrillatory activity.

7   The results also suggest that in the rat, the low currents used ($\sim 400\,\mu A$) do not release substantial quantities of catecholamines whereas these may be released by coronary artery ligation.

Introduction

Coronary artery ligation in the anaesthetized rat results in severe arrhythmias during the initial 30 min postligation period (Clark, Foreman, Kane, McDonald & Parratt, 1980). A number of known antiarrhythmic agents including membrane stabilizing agents, β-adrenoceptor blocking drugs, agents which prolong action potential duration and drugs which inhibit transmembrane calcium movement have been shown to reduce the severity of the arrhythmias in this model (Clark et al., 1980; Kane & Winslow, 1980; Kane, McDonald & Parratt, 1981; Marshall, Muir & Winslow, 1981a,b; Fagbemi & Parratt, 1981). Using Vaughan Williams' (1974) clasification, these agents will be referred to as class I, II, III and IV respectively. This model would therefore appear to be a suitable, simple and inexpensive method for studying the effects of drugs on postinfarction arrhythmias. The incidence of ventricular fibrillation (VF) following coronary artery ligation in the rat has been reported to be between 28 and 75% (Au, Collins, Harvie & Walker, 1979; Clark et al.,

1980; Kane & Winslow, 1980) and drugs are assessed for their ability to reduce the incidence of VF and the number of premature ventricular systoles (PVS). Since coronary artery ligation is known to produce an early fall in the ventricular fibrillation threshold (VFT) in the dog (Allen, James, Kelly, Shanks & Zaidi, 1977) we have applied this technique to the rat with a view to more rapidly assessing antifibrillatory activity. VF is produced in all the rats, the method obviates the laborious task of counting numbers of PVS and each animal serves as its own control. Thus fewer animals are required to give a statistically significant result.

Also, confident diagnosis of spontaneous postligation ventricular fibrillation in the rat, especially where reversion to normal sinus rhythm occurs, is made difficult because of high heart rates, often complete fusion of the QRS and T waves after ligation and often the presence of large R waves in the Lead II ECG during 'fibrillation' (Marshall et al., 1981b). Electrically-induced fibrillation results in a

Table 1   Effects of drugs on the ventricular fibrillation threshold (VFT) of the anaesthetized rat

Drug	Dose (mg/kg)	n	VFT (μA) pre	post	Mean % change	Class
Distilled water		18	453±21	441±35	−2.6	
Org 6001	5	8	421±42	444±59	+5.5	I
	10	7	406±45	**593±71	+46.1	
Bepridil	2	8	314±31	293±36	−6.7	I+IV
	5	7	337±18	**534±39	+58.5	
Metoprolol	1	7	369±27	409±32	+10.8	II
	5	7	369±27	369±29	0	
Sotalol	10	8	473±49	525±66	+11	II+III
	50	8	473±49	*672±71	+42.1	
Melperone	2.5	6	385±58	393±61	+2.1	III
	5	8	344±39	406±55	+18	
	10	8	303±43	**481±76	+58.7	
Nifedipine	0.01	8	455±37	476±59	+4.6	IV
	0.02	8	397±42	450±47	+13.4	

Results are expressed as the mean±s.e.mean of n observations. Significant differences between pre and post drug values are denoted by *($P<0.05$) and **($P<0.01$). Measurements were made prior to and 20 min after drug administration.

sustained low amplitude wave pattern more similar to fibrillation seen in higher animals.

We have therefore screened agents with differing electrophysiological profiles for their ability both to raise VFT of normal myocardium and to prevent the fall in VFT consequent upon coronary artery ligation. Some interesting differences were observed between drug effects on normal and ischaemic myocardium and between their ability to prevent the postligation fall in VFT and the reported effectiveness of some of these agents in reducing the severity of postinfarction arrhythmias in this species. Some of the possible implications of these findings are discussed.

Methods

Male Wistar rats (300–350 g) were anaesthetized with pentobarbitone sodium (60 mg/kg, 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 a Lead II electrocardiogram (ECG) recorded from subcutaneous steel needle electrodes. BP and the ECG were displayed on a Mingograph 82 ink jet recorder (Elema-Schonander). Ribs 2, 3 and 4 were cut close to the left side of the sternum to expose the heart and the chest walls retracted. Two platinum electrodes (6 mm apart) embedded in a narrow rubber strip were positioned on the left ventricular anterior wall such that the anode was approximately 3 mm below the AV ring and the cathode on or near the apex. The electrode was held in place using a rack and pinion adjustable clamp. Square wave pulses (0.8 ms duration; 50 Hz) delivered from a Nihon Kohden (SEN-

1101) stimulator were passed through a constant current stimulus isolation unit (SS-101J) to give an initial current intensity of 100 μA. Current intensity was then increased at a rate of 10 μA/s until VF developed. Drugs or vehicle were given intravenously 5 min after the control determination and VFT redetermined 20 min later.

In a separate group of animals, a 6/0 silk suture was placed under the main left coronary artery as described by Selye, Bajusz, Grasso & Mendell (1960) and a stabilization period of 15 min allowed. Drugs or vehicle were given 5 min after VFT determination. Coronary artery ligation was performed 15 min later and VFT redetermined 3 to 3.5 min postligation.

Significant differences were determined using a paired t test.

Drugs used were Org 6001 hydrochloride (Organon), metoprolol (AB Hassle) sotalol hydrochloride (Mead-Johnson), melperone hydrochloride (Ferrosan AB), nifedipine (Bayer) and bepridil hydrochloride (Organon International).

With the exception of nifedipine all drugs were dissolved in distilled water. A 6 mg/ml solution of nifedipine dissolved in equal volumes of ethanol and polyethylene glycol was prepared and further dilutions made with distilled water. Precautions were taken to avoid contact of nifedipine solutions with light.

Results

Effects of drugs on ventricular fibrillation threshold of normal myocardium

In control animals, successive determinations of VFT

Table 2 Effects of drugs on arterial blood pressure (systolic/diastolic) and heart rate

Drug	Dose (mg/kg)	n	Arterial blood pressure (mmHg) pre	post	Heart rate (beats/min) pre	post	Mean % change
Distilled water		18	106± 7/ 81± 6	103± 6/ 77± 6	427± 9	386±12***	−9.6
Org 6001	5	7	113± 7/ 83± 6	116± 9/ 83± 8	380±23	363±23	−4.5
	10	7	118± 9/100± 9	114±14/ 93±13	409±15	342±15***	−16.4
Bepridil	2	7	85± 6/ 64± 6	98± 6/ 61±11	354± 9	295±15***	−16.7
	5	7	148± 6/126± 9	131±10/100± 8*	454±18	350±20**	−22.9
Metoprolol	1	7	117± 8/ 86± 9	118± 8/ 86± 8	375± 8	309± 7***	−17.6
	5	7	120± 8/ 85± 7	109± 9/ 73± 8	375± 8	290± 9***	−22.7
Sotalol	10	8	124±10/ 98±11	*** 98± 8/ 77± 8***	382±15	248±12***	−35.1
	50	8	126±10/100±11	*** 68± 6/ 44± 5***	387±14	207±13***	−46.5
Melperone	2.5	6	123±13/103±13	** 75± 5/ 60± 3*	403±12	333±13**	−17.4
	5	8	150±11/119±10	*** 87± 6/ 58± 6***	453±12	371±17***	−18.1
	10	9	133±10/109± 8	*** 73± 7/ 52± 5***	417± 9	284±12***	−31.9
Nifedipine	0.01	8	99±11/ 76±11	106± 7/ 80± 8	388±19	357±23	−8.0
	0.02	8	96± 9/ 83± 9	105± 8/ 90± 9	398±10	380±19	−4.5

Results are expressed as the mean ± s.e.mean of n observations. Significant differences between predrug values and values recorded 20 min after administration are denoted by *($P<0.05$), **($P<0.01$) and ***($P<0.001$).

were similar. The effects of Org 6001 (class I), metoprolol (class II), sotalol (class II and III), melperone (class III), nifedipine (class IV) and bepridil (class I and IV) on VFTs are summarized in Table 1. Org 6001 (10 mg/kg), sotalol (50 mg/kg), melperone (10 mg/kg) and bepridil (5 mg/kg), produced a significant increase in VFT. Lower doses of these agents failed to change VFT significantly and metoprolol (1–5 mg/kg) and nifedipine (10–20 μg/kg) were also ineffective at the doses used.

Effects on heart rate and blood pressure

Table 2 lists values of arterial blood pressure and heart rate recorded prior to and 20 min after drug administration. Org 6001, metoprolol and bepridil induced transient reductions in blood pressure and by 20 min, values were not different from those recorded prior to drug administration. In contrast, sotalol and melperone at all doses used, caused marked and sustained decreases in blood pressure. Nifedipine produced only a modest and very transient effect on arterial pressure.

With the exception of nifedipine, all drugs used induced a fall in heart rate which was particularly marked in response to sotalol and the high dose of melperone.

Effects of drugs on the fall in VFT induced by coronary artery ligation

The results are summarized in Table 3. In control animals VFT fell by a mean of 28% after 3 to 3.5 min of coronary artery occlusion. Doses of Org 6001

(5 mg/kg), metoprolol (5 mg/kg), sotalol (10 mg/kg) and bepridil (2 mg/kg), which failed to increase VFT of normal myocardium either prevented or substantially reduced the fall in VFT consequent upon coronary artery ligation. Nifedipine (10 μg/kg) and the low dose of melperone (2.5 mg/kg) failed to prevent the decrease in VFT. The highest dose of melperone used (10 mg/kg) increased VFT to a similar extent to that observed in normal myocardium.

Discussion

The results of the present study indicate that drugs possessing a membrane stabilizing action such as Org 6001 (Salako, Vaughan Williams & Wittig, 1976) or bepridil (Kane & Winslow, 1980) or drugs which prolong the cardiac action potential duration, e.g. melperone (Olsson & Arlock, 1976) and sotalol cause an increase in VFT of normal rat myocardium whereas those with a selective cardiac β_1-adrenoceptor blocking action (metoprolol) (Ablad, Carlsson, Dahlof & Ek, 1975) or a specific calcium antagonistic effect (nifedipine) (Fleckenstein, Fleckenstein-Grun, Byon, Haastert & Spah, 1979) fail to raise VFT. In doses required to increase VFT, Org 6001 and bepridil reduced heart rate by 16 and 32% respectively. However, doses of metoprolol (5 mg/kg) and sotalol (10 mg/kg) sufficient to reduce heart rate by 18 and 35% respectively failed to increase VFT significantly. It therefore seems unlikely that bradycardia *per se* induced by class I or III agents is responsible for their effects on fibrillation thresholds. Electrically induced ventricular fibrilla-

Table 3 Effects of drugs on the postligation fall in ventricular fibrillation thresholds (VFT)

Drug	Dose (mg/kg)	n	VFT (µA) Predrug	VFT (µA) Postligation	Mean decrease in VFT (µA)
Distilled water		19	405 ± 31	$***292 \pm 29$	113 ± 23
Org 6001	5	7	349 ± 45	301 ± 49	$48 \pm 26\dagger$
Bepridil	2	11	426 ± 57	354 ± 37	72 ± 55
Metoprolol	5	9	360 ± 18	341 ± 32	$19 \pm 43\dagger$
Sotalol	10	9	367 ± 42	314 ± 34	53 ± 40
	50	7	361 ± 23	341 ± 36	$20 \pm 31\dagger$
Melperone	2.5	7	388 ± 34	$**230 \pm 29$	158 ± 33
	5	8	493 ± 30	462 ± 43	$31 \pm 29\dagger$
	10	7	346 ± 32	$*589 \pm 63$	$+243 \pm 89\dagger\dagger$
Nifedipine solvent	1 ml/kg	8	344 ± 40	$*253 \pm 22$	91 ± 42
Nifedipine	0.01	8	369 ± 32	$*249 \pm 63$	120 ± 57

Results are expressed as the mean $\pm$ s.e.mean of n observations. Significant differences between values recorded 5 min prior to drug administration and 3–3.5 min after ligation are denoted by $*(P < 0.01)$, $**(P < 0.01)$ and $***(P < 0.001)$. $\dagger(P < 0.05)$ and $\dagger\dagger(P < 0.01)$ denote significant differences between changes in VFT in control and drug-treated animals.

tion (VF) would appear to be a consequence of local re-entry. Single stimuli or trains of stimuli of sufficient intensity to precipitate VF or multiple ventricular extrasystoles evoke continuous fractionated electrical activity which bridges diastole and which is seen immediately prior to the development of arrhythmias (Euler & Moore, 1980; Harumi, Smith, Abildskov, Burgess, Lux & Roland, 1980). Properties expected to break or prevent the development of re-entrant circuits include a slowing of conduction, an increase in membrane threshold potential and prolongation of the refractory period. Class I agents may exert all of these actions and class III agents also increase the duration of the cardiac refractory period.

The results obtained in the present study using class I and IV agents are similar to those obtained by others using *in vivo* techniques to determine VFT or the multiple response extrasystole threshold (MRET) of normal myocardium, e.g. class I agents increase VFT in the dog (Allen *et al.*, 1977), and cat (Szekeres & Papp, 1971) and increase MRET in the conscious rabbit (Thormahlen & Rohte, 1981). Nifedipine does not increase VFT in the dog (Bergey, McCallum & Nocella, 1981) and verapamil has little effect on MRET in the rabbit. As far as we are aware, there are no reports in the literature of the effects of class III agents on VFT *in vivo*. However, hearts taken from rats pretreated with the class III agent (Singh & Vaughan Williams, 1970) amiodarone for 3–7 days show an increase in VFT compared to control hearts (Lubbe, McFadyen, Muller, Worthington & Opie, 1979).

Our results using the class II agent metoprolol are at variance with the reported effects of other β-adrenoceptor blocking agents on VFT. Propranolol has been reported to produce a modest increase in

MRET in the conscious rabbit (Thormahlen & Rohte, 1981) and to raise VFT of cat myocardium (Szekeres & Papp, 1971) while timolol increases VFT in the dog heart (Hodess, Spear & Moore, 1979). However, trains of stimuli used to induce VF in the dog may evoke a local release of catecholamines (Euler, 1980; Winkle, Jaillon, Griffen & Schnittger, 1980) which would tend to increase the susceptibility of the heart to fibrillation. β-Adrenoceptor blockade might therefore be expected to antagonize the effects of released noradrenaline, resulting in an increase in VFT. Since metoprolol failed to raise VFT in the present experiments, it is likely that the relatively low currents used to precipitate fibrillation ($\sim 400 \mu A$ compared to 10 mA or more in larger animals) were not sufficient to release catecholamines. The increase in VFT produced by the β-adrenoceptor blocking agent, sotalol in the present experiments, probably resides in its class III action.

In the rat, coronary artery ligation results in a marked fall in VFT (Marshall *et al.*, 1981a). In the present experiments, drugs possessing a membrane stabilizing action (Org 6001 and bepridil) reduced this effect. Other class I agents, e.g. lignocaine, mexiletine and disopyramide have also been shown either to prevent or reverse the postligation fall in VFT in the dog (Allen *et al.*, 1977; Hori, Okamoto, Hatani, Imai, Toh & Takano, 1981). These effects of Org 6001 and bepridil were observed at doses which had no effects on VFT of normal myocardium. Vaughan Williams (1978) has shown that ischaemic conditions increase membrane stabilizing activity, an observation which may account for this apparently greater sensitivity of ischaemic tissue to the antifibrillatory actions of Org 6001 and bepridil.

Metoprolol also prevented the postligation fall in VFT in a dose that did not increase VFT of the non-ischaemic ventricle. A large body of evidence has accumulated to suggest that in both animals and man, myocardial infarction (MI), especially anterior MI, is accompanied by an increase in sympathetic outflow (for a review, see Ceremuzynski, 1981) which may precipitate or exacerbate the development of arrhythmias. Moreover, catecholamine depletion from myocardial adrenergic nerve terminals has been shown to occur within 30 min of coronary artery ligation in the rat (Holmgren, Abrahamson, Almgren & Erikson, 1981) and intravenous catecholamine infusion in the dog is known to induce an initial decrease in VFT of 4 min duration (Szekeres & Papp, 1971). It therefore seems likely that catecholamines are locally released at the onset of ischaemia in the rat (as thought to be the case in other species) which would contribute to the reduction in VFT. β-Adrenoceptor blockade would therefore be expected to reduce the fall in VFT evoked by coronary artery ligation.

As far as we are aware, there is no information available on the effects of the class III agent, melperone, on VFT of ischaemic myocardium. This agent has however been shown to reduce the severity of postligation-induced arrhythmias in the rat (Kane et al., 1981) at an intravenous dose of 10 mg/kg. In the present study, this dose not only prevented the fall in VFT resulting from ligation but was the only drug tested which resulted in a value of VFT following ligation which was significantly higher than the control value. Lubbe et al., (1979) reported a similar effect in rat isolated perfused hearts taken from animals chronically dosed with amiodarone. A lower dose of melperone (5 mg/kg), sufficient to prevent the postligation fall in VFT, also modestly increased VFT of normal myocardium although the result did not reach statistical significance. These pronounced effects of melperone probably reflect the ability of this agent to prolong cardiac refractory periods (Refsum, Platou & Myhre, 1981). Melperone also possesses some class I activity (Millar & Vaughan Williams, 1982) which may further contribute to lengthening of refractory periods.

The class IV agent nifedipine failed to prevent the postligation fall in VFT in a dose reported to reduce the severity of postligation arrhythmias in the rat in the absence of marked or sustained bradycardia or hypotension (Fagbemi & Parratt, 1981). Thandroyen, Higgenson & Opie (1980) found that nifedipine increased VFT of rat isolated perfused heart and attenuated the fall in VFT after ligation but only at concentrations close to those producing disturbances of AV conduction. In contrast, Daugherty & Woodward (1981) reported that nifedipine did not reduce the incidence of ventricular tachycardia or

fibrillation evoked by coronary artery ligation in rat isolated heart and Verrier & Lown (1980) also failed to demonstrate a protective effect of nifedipine against ischaemia-induced arrhythmias in the dog. The doses of nifedipine used in the present study induce only modest and very transient reductions in arterial pressure and left ventricular dP/dt max (decreases of 12 and 5% respectively at a dose of 10 µg/kg and 17 and 19% respectively at a dose of 20 µg/kg) (unpublished observations). Overall therefore it seems unlikely that the antiarrhythmic effect in the rat observed by Fagbemi and Parratt is due to a direct electrophysiological action and it is difficult to reconcile the lack of protection seen in the present study with these results. It is worthy of note that verapamil has been shown to exacerbate postligation-induced arrhythmias in this model (Kane et al., 1981).

Although all drugs possessing class I, II or III actions were effective in preventing the postligation fall in VFT, the more selective class III agent, melperone, also induced marked and sustained hypotension even in a dose which did not exert antiarrhythmic activity (2.5 mg/kg). The class II agent, sotalol, which also exerts class III actions induced sustained hypotension and pronounced bradycardia. In contrast, drugs possessing a class I action (Org 6001 and bepridil) exerted much less marked cardiovascular effects. These drugs induced transient hypotension and bepridil caused a moderate bradycardia, an action probably related to its calcium antagonistic actions (Vogel, Crampton & Sperelakis, 1979). The selective β_1-adrenoceptor blocking agent, metoprolol was essentially without effect on arterial pressure while producing a moderate reduction in heart rate. These results suggest that class I and II agents exert antifibrillatory actions in doses which do not depress cardiovascular function whereas the antiarrhythmic effects of agents possessing a class III action are associated with cardiovascular depression.

In conclusion, comparison of fibrillation thresholds prior to and after coronary artery ligation in the anaesthetized rat constitutes a simple, rapid and inexpensive screen for class I, II and III antiarrhythmic agents. The results obtained appear to correlate well with those observed by other workers using larger animals and other indices of protection against ischaemia-induced arrhythmias.

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