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Independent contribution of catecholamines to arrhythmogenesis during evolving infarction in the isolated rat heart

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1 Ventricular fibrillation (VF) in conscious rats with coronary artery ligation occurs in two phases, before (phase 1) and after (phase 2) 90 min of ischaemia respectively. The mechanisms of phase 2 VF are not established. Interestingly, phase 2 VF is absent in isolated (denervated) buffer-perfused rat hearts. We investigated whether catecholamine supplementation (to mimic sympathetic drive) was sufficient to restore phase 2 VF in such hearts.

2 Isolated rat hearts ($n=10$ per group) underwent coronary ligation for 240 min. At 90 min, during a period of relative electrical stability, the perfusion solution was switched from standard (Krebs) to identical solution or Krebs containing catecholamines (313 nM noradrenaline and 75 nM adrenaline) with or without 10 μM trimazosin (an α_1-adrenoceptor antagonist) or 10 μM atenolol (a β_1-adrenoceptor antagonist).

3 Although in all groups the incidence of phase 1 VF was high (80–100%), the temporal distribution of VF was monophasic, i.e. only one heart in one group developed phase 2 VF ($P=$NS). Other ventricular arrhythmias (e.g., tachycardia; VT) exhibited a similar temporal distribution. Nevertheless, haemodynamic changes confirmed sympathomimetic effects of catecholamines, e.g., heart rate was increased from 278 ± 7 beats min^{-1} in controls to 335 ± 8 beats min^{-1} ($P<0.05$) by catecholamines, an effect that could be blocked by atenolol (285 ± 7 beats min^{-1}) but not by trimazosin (342 ± 12 beats min^{-1}). Coronary flow was correspondingly increased from 7.7 ± 0.7 ml min^{-1} g^{-1} to 16.5 ± 1.3 ml min^{-1} g^{-1} ($P<0.05$); this effect could be blocked by atenolol (8.1 ± 0.6 ml min^{-1} g^{-1}) and was enhanced by trimazosin (20.7 ± 2.4 ml min^{-1} g^{-1}).

4 In conclusion, despite evidence of adequate α- and β-adrenoceptor activation, catecholamine supplementation to isolated buffer-perfused rat hearts was insufficient to restore phase 2 VF. It therefore appears unlikely that catecholamines alone mediate phase 2 VF.

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Abbreviations: DMSO: dimethyl sulphoxide; ECG: electrocardiogram; SCD: sudden cardiac death; VF: ventricular fibrillation; VPBs: ventricular premature beats; VT: ventricular tachycardia

Introduction

Currently there are no effective drug treatments to prevent sudden cardiac death (SCD) resulting from ventricular fibrillation (VF) (CAST, 1989; Waldo *et al*., 1996). *In vivo*, animals subjected to permanent coronary artery ligation exhibit two distinct phases of VF. Phase 1 VF (which may itself have subcomponents) occurs during the first 30 min of ischaemia and, following a period of relative electrical stability, phase 2 VF occurs usually between 2 and 4 h after the onset of ischaemia, during the period when infarction is developing (Curtis *et al*., 1987; Harris, 1950; Opitz *et al*., 1995). In contrast, *in vitro* preparations (such as the buffer-perfused rat heart), which are devoid of sympathetic innervation, do not exhibit phase 2 VF, despite unequivocal evidence of infarct development during sustained (4 h) ischaemia (Ravingerova *et al*., 1995). This suggests that the two phases of VF may not only be dissociable temporally, but also distinct in terms of their underlying mechanisms. It is our hypothesis that mediators released as the infarct is evolving are responsible for phase 2 VF.

Most research in animals has focussed on the mechanisms of phase 1 VF, which are now thought to involve (at the electrophysiological level) re-entry and flow of injury current (Janse, 1991), and (at the biochemical level) the actions of regional hyperkalaemia (Curtis, 1991) and various other intercellular biochemicals such as platelet activating factor (Curtis *et al*., 1993b). Since phase 1 VF is common in isolated (denervated) perfused hearts (Curtis, 1998), phase 1 VF can be assumed to be essentially independent of the autonomic nervous system. However, the absence of phase 2 VF in isolated, denervated rat hearts (Ravingerova *et al*., 1995) suggests that the presence of intact sympathetic innervation, and the action of locally released catecholamines, may be necessary for its occurrence.

It is known that there is significant catecholamine release within ischaemic hearts *in vivo* (Lameris *et al*., 2000), and a

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similar release is seen *in vitro* (Schomig *et al*., 1984). However, a diminished β-adrenergic responsiveness is observed approximately 1 h after the onset of ischaemia (Vatner *et al*., 1988), as infarction evolves. It would appear, therefore, that if catecholamines are important for phase 2 arrhythmogenesis, their site of action is likely to be non-ischaemic tissue and/or the zone between ischaemic and non-ischaemic tissue. In connection with this, there has been renewed interest in the contribution of catecholamines to phase 2 arrhythmias following the observation that, in canine epicardial border-zone tissue, β-adrenergic receptors lose the ability to desensitize 6 h after induction of ischaemia following a 2-stage coronary ligation protocol (Yu *et al*., 2000).

It would appear therefore, that there is a need to examine more directly the role of locally released catecholamines in the initiation of phase 2 VF. In the present study, the action of a variety of interventions on the temporal distribution of arrhythmias was examined in the rat heart subjected to permanent coronary occlusion *in vitro*, to determine whether exogenous catecholamine supplementation (to mimic sympathetic drive to the non-ischaemic region) is sufficient to restore phase 2 VF. Selective antagonists were used to identify the receptors involved. The results have been presented in part to the British Pharmacological Society (Clements-Jewery & Curtis, 2001).

Methods

Animals and general experimental methods

All experiments were performed in accordance with the United Kingdom Home Office *Guide on the Operation of the Animals (Scientific Procedures) Act 1986*. The perfusion technique, methods for induction of ischaemia, methods for verification and quantification of ischaemic zone size, and the techniques for recording, quantifying, and analysing data were as described previously (Curtis & Hearse, 1989b).

Rats (male Wistar; Bantin and Kingman U.K., 240–310 g; $n=10$/group) were anaesthetized with pentobarbital (60 mg kg^{-1} i.p.) and heparinized with 300 I.U heparin i.v. to prevent blood clot formation in the coronary vasculature. Hearts were excised and placed into ice-cold solution containing (in mM): NaCl 118.5, $NaHCO_3$ 25.0, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.4, KCl 3.0 and glucose 11.1, then perfused in the Langendorff mode, with perfusion solutions delivered at 37°C, pH 7.4 and at a pressure of 80 mmHg. Perfusion solutions were not recirculated. The temperature of the perfusate was monitored by insertion of a probe (Hanna Instruments, Merck U.K. Ltd) into the pulmonary conus. All solutions were filtered (5 μm pore size) before use. A unipolar electrocardiogram (ECG) was used for recording and assessment of arrhythmias. A traction-type coronary occluder consisting of a silk suture (Mersilk, 4/0) threaded through a polythene guide was used for coronary occlusion. The suture was positioned loosely around the left anterior descending coronary artery beneath the left atrial appendage. Regional ischaemia was induced by tightening the occluder.

Experimental protocol

Hearts were perfused for an initial 10 min with control perfusion solution (composition as described above), and then made regionally ischaemic for 240 min by occlusion of the left main coronary artery. After 90 min of ischaemia, the perfusion solution was switched in a randomized, blinded manner from standard solution to one of several test solutions (Table 1), which were perfusion solutions containing catecholamines alone (313 nM noradrenaline plus 75 nM adrenaline), catecholamines plus 10 μM trimazosin (an α_1-adrenoceptor antagonist) or catecholamines plus 10 μM atenolol (a β_1-adrenoceptor antagonist), with two control groups (see below). Five μM ascorbate was added to all catecholamine solutions to limit the extent of their oxidation (Hearse & Sutherland, 1999). Dimethyl sulphoxide (DMSO) 0.05% v v^{-1} was used as drug vehicle for trimazosin, and so was added to all catecholamine solutions to eliminate the vehicle as a source of variance. The two control groups included in the study design were therefore: a drug vehicle control group (perfusion solution plus water and DMSO) and an antioxidant control group (perfusion solution plus water, DMSO and ascorbate). Blinding was achieved by coding the solutions, with the code unknown to the experimenter. The choice of coded perfusion solution for each heart was made by reference to a randomization table.

Measurement of ischaemic zone size and coronary flow

At the end of the experiment the extent of the ischaemic zone was quantified using the disulphine blue dye exclusion method (Curtis & Hearse, 1989b) and expressed as per cent of total ventricular weight. Coronary flow was measured by timed collection of coronary effluent. Values of coronary flow in the ischaemic and non-ischaemic regions were calculated from the total coronary flow and the weights of the regions, as described previously (Curtis & Hearse, 1989b). Flow was calculated as ml min^{-1} g^{-1} of wet tissue, thus taking into account any differences in weight between individual hearts.

Arrhythmia diagnosis and ECG analysis

The ECG was recorded using a MacLab system, and was used to assess arrhythmias in accordance with the Lambeth Conventions (Walker *et al*., 1988). No attempt was made to defibrillate hearts if VF occurred. Ventricular premature beats (VPBs) were defined as premature QRS

Table 1 Composition of the different test solutions introduced at 90 min of ischaemia

Solution	*Composition*
Vehicle control	Krebs plus DMSO (0.05% v v^{-1}), water
Antioxidant control	Krebs, DMSO, water, 5 μM ascorbate
Catecholamines	Krebs, DMSO, water, 5 μM ascorbate, 313 nM noradrenaline, 75 nM adrenaline
Catecholamines with β_1-blocker	Krebs, DMSO, water, 5 μM ascorbate, 313 nM noradrenaline, 75 nM adrenaline, 10 μM atenolol
Catecholamines with α_1-blocker	Krebs, DMSO, water, 5 μM ascorbate, 313 nM noradrenaline, 75 nM adrenaline, 10 μM trimazosin

complexes occurring independently of a P wave, and hence included individual VPBs, bigeminy and salvos as defined by the Lambeth Conventions. The number of VPBs occurring during a specified time period in each heart was $\log_{10}$ transformed to produce a Gaussian-distributed variable for calculation of group mean values (Curtis *et al*., 1987). QT intervals at the point of 90% repolarization (QT_{90}), heart rate and PR intervals were also measured from the ECG, as previously described (Ridley *et al*., 1992). The ECG was recorded at a sampling rate of 1 kHz, allowing millisecond precision for measurement of ECG intervals. Measurement of all variables was performed in a blinded manner.

Exclusion criteria

A set of exclusion criteria, based on those used previously (Curtis *et al*., 1993a; Ridley & Curtis, 1992), were modified for the study of phase 2 arrhythmias. Any heart with a sinus rate of <250 beats min^{-1} prior to coronary ligation, or a coronary flow >18 ml min^{-1} g^{-1} or <8 ml min^{-1} g^{-1} at 1 min before the onset of ischaemia, or with an ischaemic zone of $<30\%$ or $>50\%$ of total ventricular weight, was excluded. Any heart displaying atrio-ventricular dissociation in both pre-90 and post-90 min time periods was also excluded on the basis of intrinsic atrio-ventricular node abnormalities. It was intended that if any heart remained in VF at 90 min after the start of ischaemia, it would be replaced. However, self-terminating VF is typical in rat hearts (Curtis, 1998) and, despite a high incidence of phase 1 VF, all episodes had self-terminated by 90 min (individual episodes lasted from 1 to 930 s) precluding the need to exclude and replace hearts on this basis. The total numbers of hearts excluded in each group are given in Table 2.

Rationale for choice of drug concentrations

The concentrations of noradrenaline (313 nM) and adrenaline (75 nM) were calculated from an estimate (1 μg min^{-1} of noradrenaline, and 0.25 μg min^{-1} of adrenaline) of the respective amounts needed to restore heart rate to values similar to those encountered in conscious rats (Curtis *et al*., 1985). The concentration ratio is within the range encountered in man, especially under conditions of stress (Baumgartner *et al*., 1985; Coplan *et al*., 1989; Ratge *et al*., 1986). The concentrations of the antagonists were chosen to be 1–2 orders of magnitude above their respective pA_2 values for cardiac tissue [7.25 for atenolol (Kenakin, 1993) and 6.62 for trimazosin (Constantine & Hess, 1981)] yet within the limits of their selectivity for β_1- and α_1-adrenoceptors respectively. The chosen concentration of atenolol was shown to abolish the positive chronotropic effects of the above concentrations of noradrenaline and adrenaline in preliminary studies. Although trimazosin appears to have some vasodilatory properties independent of α_1-adrenoceptor antagonism, these effects only become significant at concentrations approximately 5–10 times the concentration used in the present experiments (Constantine *et al*., 1984). Importantly, both drugs are free of Na^+ channel blocking activity (Daugherty *et al*., 1986). The drugs and the chosen concentrations were therefore considered to be appropriate for specific and selective probing of the role of α_1- and β_1- receptors in mediating an arrhythmogenic effect of catecholamine perfusion. All chemicals were dissolved in water, except for trimazosin, which was dissolved in DMSO. The final concentration of DMSO in the perfusion solutions was 0.05% v v^{-1}. The concentration of ascorbate used was based on the ratio of noradrenaline to ascorbate concentrations used previously in related studies in perfused hearts (Hearse & Sutherland, 1999).

Statistics

Gaussian distributed variables (expressed as mean $\pm$ s.e.m.), were subjected to analysis of variance followed by Dunnett's or Tukey's tests where appropriate. Binomially distributed variables were compared using Mainland's contingency tables (Mainland *et al*., 1956) as previously described (Curtis & Hearse, 1989a). $P<0.05$ was taken as indicative of a statistically significant difference between values. Arrhythmia incidences were expressed as the percentage of hearts in each group experiencing each arrhythmia during consecutive time intervals, in order to reveal the temporal pattern of arrhythmogenesis.

Drugs and materials

($\pm$)-Noradrenaline, ($-$)-adrenaline, atenolol and ascorbate were all obtained from Sigma Chemicals (U.K.). Trimazosin was a gift from Pfizer (U.K.). All salts were reagent grade chemicals obtained from Sigma Chemicals (U.K.). Water for preparing perfusion solution was supplied using a reverse osmosis system (USF Elga Ltd, U.K.), and had a specific resistivity of greater than 18 MΩ.

Table 2 Numbers of excluded hearts in each group and the reasons for their exclusion

	Number of hearts excluded in each group				
Reason for exclusion	*Drug vehicle control*	*Anti-oxidant control*	*Catecholamines alone*	*Catecholamines plus atenolol*	*Catecholamines plus trimazosin*
Ischaemic zone size $>50\%$	3	1	2	1	3
Ischaemic zone size $<30\%$	1	1	0	0	1
Coronary flow <8 ml min^{-1} g^{-1} 1 min before ligation	0	3	1	0	0
Coronary flow >18 ml min^{-1} g^{-1} 1 min before ligation	1	0	0	1	0
Heart rate <250 beats min^{-1} before ligation	1	1	0	2	0
Atrio-ventricular dissociation in both pre- and post-90 min periods	0	1	2	0	1
Phase 1 VF persisting to 90 min	0	0	0	0	0

All excluded hearts were replaced to maintain equal group sizes.

Results

Haemodynamic and ECG changes

Haemodynamic changes following switch of solutions confirmed sympathomimetic effects of catecholamines, such that after 30 min of perfusion, heart rate (Figure 1) was increased from 278 ± 7 beats min^{-1} in controls to 335 ± 8 beats min^{-1} ($P<0.05$), an effect that could be blocked by atenolol (285 ± 7 beats min^{-1}) but not by trimazosin (342 ± 12 beats min^{-1}). Coronary flow (Figure 2) was correspondingly increased from 7.7 ± 0.7 ml min^{-1} g^{-1} to 16.5 ± 1.3 ml min^{-1} g^{-1} ($P<0.05$); this effect could be blocked by atenolol (8.1 ± 0.6 ml min^{-1} g^{-1}) and was enhanced by trimazosin (20.7 ± 2.4 ml min^{-1} g^{-1}).

Coronary occlusion initially caused prolongation of the QT_{90} interval (Figure 3) in agreement with published data for this model (Rees & Curtis, 1993), from 52 ± 1 ms 1 min before occlusion to 71 ± 1 ms at 15 min of ischaemia (values from the five groups combined – there were no significant differences between groups prior to switch of solutions). Thereafter QT_{90} intervals declined to a mean of 59 ± 1 ms 1 min before switch of solutions. Catecholamines also prolonged the QT interval from 58 ± 2 ms in controls, to 69 ± 1 ms ($P<0.05$) after 30 min of perfusion. This effect was not antagonized by 10 μM atenolol (68 ± 1 ms) or by 10 μM trimazosin (66 ± 2 ms).

Catecholamines produced a shortening of the PR interval from 44 ± 1 ms in controls (to 36 ± 1 ms after 30 min of catecholamine perfusion; $P<0.05$), an effect that was antagonized by 10 μM atenolol (42 ± 1 ms) but not by 10 μM trimazosin (39 ± 1 ms; $P<0.05$ vs controls).

Arrhythmias and ischaemic zone size

Ischaemic zone sizes were similar in each group, ranging from 44 ± 1 to 40 ± 1 (P = NS; values expressed as per cent of total ventricular weight). Values are typical for rat hearts with proximal left coronary occlusion (Ridley *et al.*, 1992). This indicates that the intrinsic arrhythmogenic substrate was consistent between groups.

Ventricular fibrillation (VF)

The incidence of VF displayed a largely monophasic temporal pattern in all groups over the 240 min of ischaemia (Figure 4). The incidence in the various groups ranged from 80–100%. All VF, except one episode in one heart, occurred during the first 90 min of ischaemia (phase 1 VF), during which time all hearts were perfused with standard Krebs solution. The occurrence of new episodes of VF diminished with time after the first 30 min of ischaemia, such that by 90 min the incidence of VF during the preceding 15 min period was zero in all groups. After switch of solutions at 90 min of ischaemia, only one heart (from the group perfused with solution containing catecholamines together with trimazosin), developed a further episode of VF (P = NS).

Ventricular tachycardia (VT)

VT occurred in every heart during the first 30 min of ischaemia. Thereafter susceptibility to new episodes declined rapidly in a manner similar to that seen for VF (Figure 5). Following switch of solutions at 90 min of ischaemia, there was a second phase of VT in hearts perfused with catecholamines alone and catecholamines together with trimazosin. The appearance of VT occurred 180 min or more after the start of ischaemia. The incidence of this late VT was too low, however, for it to reach a level of statistical

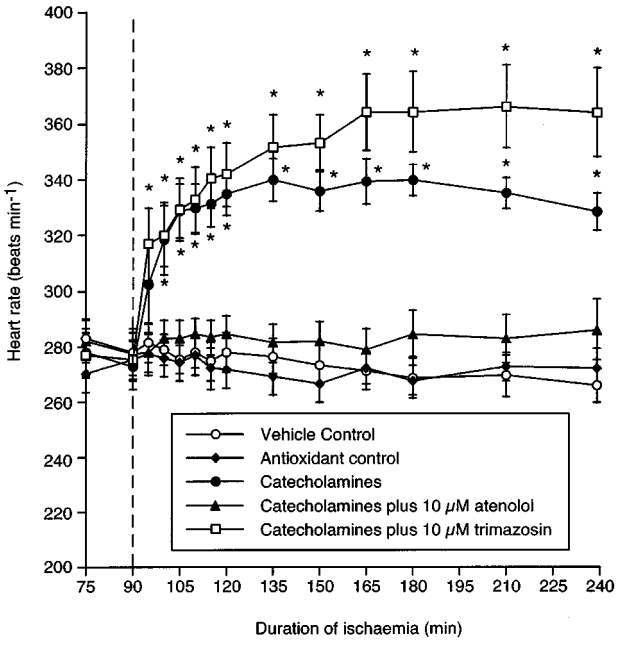

Figure 1 Heart rate (beats min^{-1}) at specific times during ischaemia. The vertical dashed line indicates switch of solutions from standard Krebs to one of the various solutions described in the figure (for full details, see Methods). *$P<0.05$ vs vehicle control.

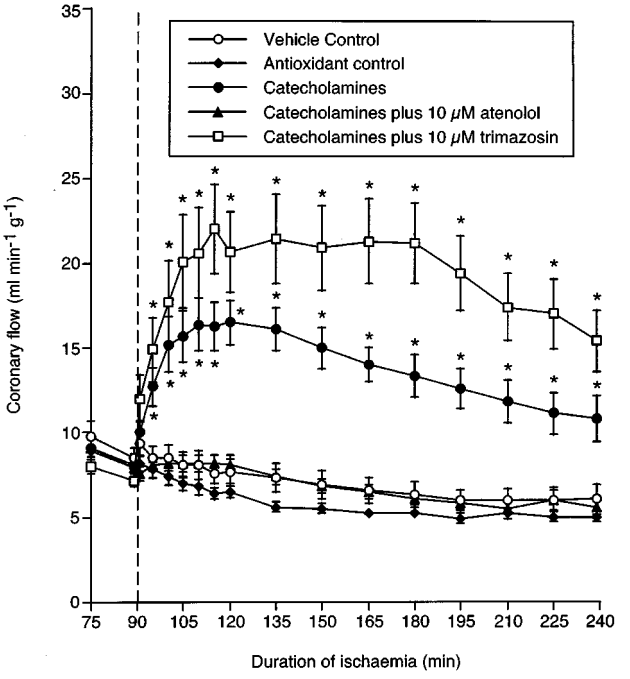

Figure 2 Coronary flow in the uninvolved region in ml min^{-1} g^{-1} at specific times during ischaemia. The vertical dashed line indicates switch of solutions as described in the legend to Figure 1. *$P<0.05$ vs vehicle control.

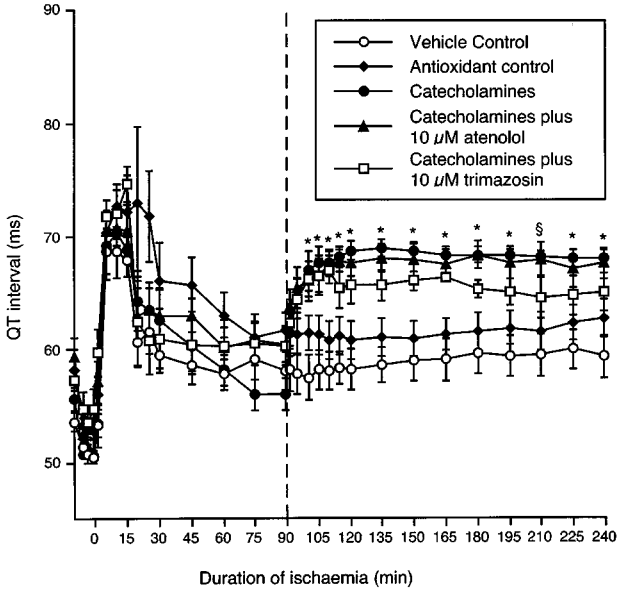

Figure 3 QT_{90} intervals (ms) at specific time points during ischaemia. The vertical dashed line indicates switch of solutions as described in the legend to Figure 1. Regional ischaemia began at 0 min. $^*P<0.05$ for the three catecholamine-containing groups vs vehicle control; § indicates $P<0.05$ for vehicle control vs catecholamines alone and catecholamines plus atenolol.

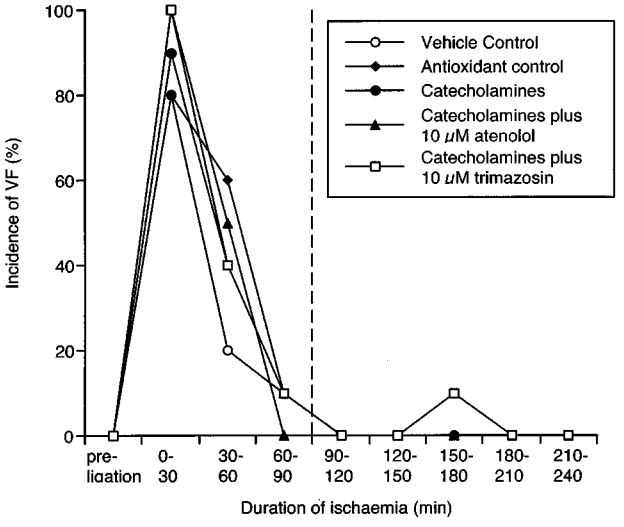

Figure 4 Incidence of VF in consecutive 30 min periods during regional ischaemia. The vertical dashed line indicates switch of solutions as described in the legend to Figure 1. Group incidences were not significantly different from each other at any time interval ($P>0.05$).

significance compared with the control group (in which late VT was absent).

Salvos

The incidence of salvos was very high during the first 30 min of ischaemia (100% in all groups), and declined thereafter, though more slowly than the incidences of VF and VT. After the solutions had been switched, new episodes of salvos

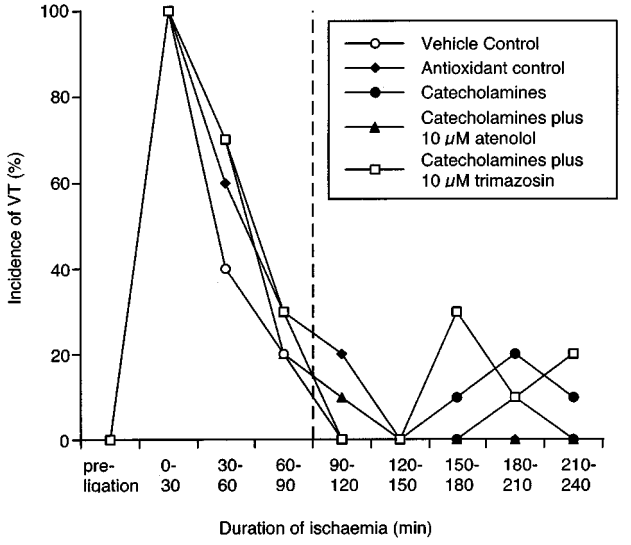

Figure 5 Incidence of VT in consecutive 30 min periods during regional ischaemia. The vertical dashed line indicates switch of solutions as described in the legend to Figure 1. Group incidences were not significantly different from each other at any time interval ($P>0.05$).

occurred. The incidence was higher (as a non-significant trend) in the groups containing catecholamines alone and catecholamines together with trimazosin compared to control over the time period 180–240 min (data not shown).

Bigeminy

The incidence of bigeminy, like that of other arrhythmias, was very high during the first 30 min of ischaemia (100% overall incidence), exhibiting a temporal pattern similar to that seen with salvos. Following switch of solutions, there were no statistically significant differences between groups, and no clear evidence of a second phase of bigeminy induced by catecholamine supplementation (data not shown).

Ventricular Premature Beats (VPBs)

The incidence (per group) of VPBs was high throughout the 240 min duration of ischaemia in all groups. The frequency (mean number) of VPBs, when $\log_{10}$ transformed, revealed a monophasic temporal pattern (Figure 6) with the frequency very high during the first 60 min of ischaemia, declining thereafter. There were no statistically significant differences between groups, and no evidence of a second phase of VPBs induced by catecholamine supplementation.

Relationship between phase 2 arrhythmias and other variables

There was no relationship between susceptibility to the arrhythmias that occurred after 90 min and any other variable (heart rate, coronary flow, PR interval, etc). For example, the QT_{90} interval and the coefficient of regression (r^2) between QT_{90} intervals and the corresponding $\log_{10}$-transformed VPB frequency in the post-90 min period, either within or between groups, was 0.002 ($P=$NS).

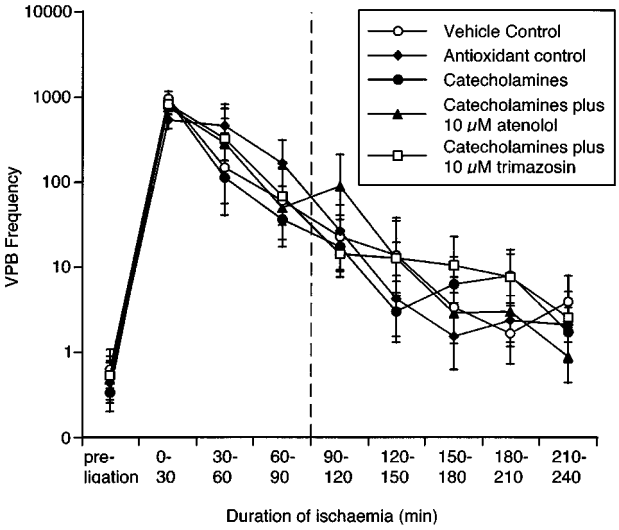

Figure 6 Frequency of VPBs in consecutive 30 min periods during regional ischaemia. Values are plotted on a logarithmic ordinate for reasons described in Methods. The vertical dashed line indicates switch of solutions as described in the legend to Figure 1. Group values are not significantly different from each other at any time interval ($P>0.05$).

Discussion

Most studies on the possible role of catecholamines in arrhythmogenesis have focussed on phase 1 arrhythmias (e.g. Bolli *et al*., 1984), or have used the Harris two-stage ligation model (Harris, 1950; Patterson *et al*., 1986). The latter is inappropriate for study of phase 2 arrhythmias since in this model the overall incidence of VF is low, and the temporal pattern of arrhythmias is disrupted by the two-stage ligation procedure. Thus a different approach was considered necessary in the present study. It was found that restoration of α- and β-adrenoceptor activation by addition of catecholamines was insufficient in buffer-perfused hearts to restore a second phase of VF and other ventricular arrhythmias to the level seen *in vivo* (Curtis *et al*., 1987) in rats with equivalent ischaemic regions (no VF versus a 100% incidence *in vivo*). Therefore an intact sympathetic nervous system would appear not to be sufficient alone to account for phase 2 VF *in vivo*. The strength of this conclusion depends on the achievement of specific and selective adrenoceptor modulation by the pharmacological tools chosen for the present study.

Evidence of cardiac adrenoceptor modulation by catecholamines and antagonists

Catecholamine perfusion had distinct pharmacological effects, increasing heart rate and coronary flow and shortening the PR interval. These effects are important because they establish that the concentration of catecholamines chosen were adequate for the intended purpose (achievement of adrenoceptor activation).

The fact that the vasodilator, tachycardic and PR-shortening effects were completely antagonised by 10 μM atenolol, a relatively selective β_1-adrenoceptor antagonist (pA_2 7.25 and $\sim$5.5 for β_1 and β_2 receptors respectively in rat tissue; Kenakin, 1993; Rimele *et al*., 1988), indicates that these effects are β_1-adrenoceptor mediated. Although some antagonism of β_2-adrenoceptors would have occurred at the concentration of atenolol used in the present experiments, the coronary vasodilatation caused by catecholamine perfusion likely resulted from the involvement of β_1-adrenoceptors alone, with both a direct action on the coronary resistance vasculature through vascular β_1-adrenoceptors (Nyborg & Mikkelsen, 1985), and an indirect vasodilatory action *via* inotropy-related increases in myocardial metabolite production (Berne & Rubio, 1979) accounting for the increase in coronary flow. Additionally, the present data also suggest that catecholamine-mediated α_1-agonism has an opposing influence on β_1-adrenoceptor-mediated coronary vasodilatation since, in the presence of trimazosin (a selective α_1-adrenoceptor antagonist), catecholamine perfusion elicited a greater coronary flow than that achieved in the absence of trimazosin. This is consistent with the known distribution of adrenoceptors in rat coronary artery; the proximal artery region is α-adrenoceptor dominated, whereas the distal resistance artery region is β-adrenoceptor dominated (Nyborg, 1990). The present results are thus consistent with the expected weak constrictor effect of noradrenaline and adrenaline in proximal coronary artery, which is antagonized by trimazosin, leading to unopposed β-mediated vasodilatation. Overall, these data confirm that catecholamine perfusion had achieved its objective (restoration of cardiac β_1- and α_1-adrenoceptor activation). Despite this, there was no concomitant restoration of phase 2 arrhythmogenesis.

The QT_{90} interval and phase 2 arrhythmias

The relationship between the QT interval and phase 2 arrhythmias is unknown (not only in rat hearts but also in other species). Its relationship to phase 1 VF in rat hearts is intriguing, and has been studied extensively (Rees & Curtis, 1995; Tsuchihashi & Curtis, 1991). In the present study, catecholamine perfusion increased the QT interval, but this effect was not significantly reduced by atenolol or trimazosin. The absence of an effect of atenolol, which contrasts with the effects it had on catecholamine-induced increases in heart rate and coronary flow, suggests that the catecholamine-induced QT widening observed during the later phase of ischaemia is not β_1-mediated. Likewise the lack of effect of trimazosin suggests that α_1-receptors are not involved. This is intriguing since α_1-agonism is known to prolong the action potential duration in isolated myocytes (Dukes & Vaughan Williams, 1984; Fedida & Bouchard, 1992). One possible explanation is that because trimazosin has a relatively low affinity for the α_1-adrenoceptor (Constantine & Hess, 1981), the catecholamine-induced QT widening could have resulted from α_1-agonism that was inadequately blocked by trimazosin. However, the enhancement of coronary flow by catecholamines in the presence of trimazosin, discussed earlier, is evidence that substantial blockade of α_1-adrenoceptors was achieved with trimazosin. Indeed, this is expected since the concentration of trimazosin used was greater than one order of magnitude above its pA_2 value for α_1-adrenoceptors (Constantine & Hess, 1981). A higher concentration of trimazosin could have been used to explore further the mechanism of catecholamine effects on the QT interval, but this would have led to loss of molecular selectivity

(Constantine *et al*., 1984), precluding the ability to attribute pharmacological effects solely to α_1-blockade.

It is important to emphasise, however, that the QT changes seen do not require comprehensive explanation, since the key observation was that the QT interval (and its modulation by catecholamines, in the presence and absence of atenolol or trimazosin) was unrelated to susceptibility to phase 2 arrhythmias. This accords with an equivalent lack of relationship between QT intervals and susceptibility to phase 1 arrhythmias in rat hearts (Rees & Curtis, 1996).

Methodological justification

As a consequence of the method utilized in the present experiments, catecholamines were delivered only to uninvolved (non-ischaemic) and border zone tissue during ischaemia. This can be regarded as pathophysiologically appropriate if the assumption is made that catecholamine release within the ischaemic zone is similar *in vitro* and *in vivo* during the period when phase 2 arrhythmias occur *in vivo*. The process of catecholamine release within ischaemic tissue has been well described and occurs in three phases. The first phase (up to 10 min of ischaemia), if present, is dependent on efferent cardiac sympathetic nerve activity, but is short-lived owing to an ischaemia-induced hyperkalaemia-related block of nerve conduction (Dart *et al*., 1984). A second phase (10–40 min of ischaemia) of release is non-exocytotic and is mediated by the neuronal noradrenaline uptake mechanism operating in the reverse mode (Schomig *et al*., 1984). A third phase (>40 min of ischaemia) is insensitive to blockers of neuronal uptake and is probably secondary to sympathetic nerve degeneration (Schomig *et al*., 1984). Thus, most catecholamine release (and all release during the period when phase 2 arrhythmias occur) is independent of efferent nerve activity. Importantly, this means that during the period when phase 2 arrhythmias occur, there is no scope for sympathetic nerve-mediated noradrenaline release, even *in vivo*. Therefore, the main difference between the model used in the present study and the *in vivo* setting is that a structurally and functionally intact sympathetic innervation is maintained to uninvolved tissue *in vivo*, but not *in vitro*. Thus the replenishment of catecholamines to uninvolved tissue *in vitro* achieved in the present study mimics the *in vivo* situation from the functional perspective.

Relevance to the efficacy of β_1-antagonists in acute myocardial infarction

In the present study, although β_1- and α_1-antagonism ameliorated distinct pharmacological effects of catecholamine perfusion in the isolated heart, neither β_1- nor α_1-antagonism had any significant effect on phase 2 arrhythmias. However, the relative absence of phase 2 arrhythmias, even in hearts supplemented with catecholamines, suggests that β_1- and α_1-antagonists have no protective effects because β_1- and α_1-agonism do not play a necessary causal role in phase 2 arrhythmogenesis. This means that if phase 2 arrhythmias have a clinical counterpart they are unlikely to be sensitive to suppression by β_1- and α_1-adrenoceptor antagonism. It is not possible to determine whether this is (or is not) the case by examination of clinical data. This is because clinical SCD data do not specify the precise relationship between the time of death and the onset of ischaemia. Therefore no clear distinctions are made between ischaemia-related and infarct-related mortality, and it is therefore not possible to ascertain whether phase 1 or phase 2 VF is responsible for individual deaths. Nevertheless, available data suggest that overall mortality (SCD plus other causes of death) is reduced by β_1-blockers (Antman *et al*., 1992; BHAT trial, 1982; ISIS-1 trial, 1986; Wilhelmsson *et al*., 1974; Yusuf *et al*., 1985). However, the reduction in total mortality is small ($14 \pm 6\%$, mean $\pm$ s.d.), the reduction in the incidence of VF is also small ($15 \pm 7\%$, mean $\pm$ s.d.), and therefore a substantial proportion of the reduction in mortality cannot be attributed to an effect on VF incidence since the effect on overall mortality is far in excess of the effect on VF (ISIS-1 trial, 1986). It is therefore unlikely that β_1-blockers have a direct effect on VF, let alone a specific effect on phase 2 VF, in man. This accords with the present findings.

Although phase 1 and phase 2 patterns of VF are well established in various animal preparations and species, it is not clear whether VF can in fact be subdivided this way in man. VF probably does occur during infarct development as well as during ischaemia because approximately 50% of patients successfully resuscitated from VF have evidence of infarction (de Vreede-Swagemakers *et al*., 1998). However, the profile of VF evolution during infarction remains insufficiently characterised (Campbell *et al*., 1981) because it arises in most patients without warning when the individual is otherwise well and out of hospital (Adgey *et al*., 1982).

If not catecholamines, what causes phase 2 VF?

The present findings indicate that factors other than catecholamines are necessary for mediating phase 2 VF. Clues to their identity may be provided by considering the mediation of phase 1 VF during early myocardial ischaemia (0–30 min). Here, the manifestation of VF represents the net outcome of an interaction between arrhythmogenic mediators and endogenous protectants (Curtis *et al*., 1993b; Parratt, 1993) and the balance is strongly in favour of the arrhythmogenic mediators both *in vivo* and in Krebs-perfused hearts *in vitro*. This balance can be shifted by certain forms of manipulation such as ischaemic preconditioning which increase the production of putative endogenous protectants such as nitric oxide and calcitonin gene-related peptide (Ferdinandy *et al*., 1997).

The identity of many of the likely candidates that determine VF susceptibility during early ischaemia is known (Curtis *et al*., 1993b; Parratt, 1993). However, there is scant information (of even qualitative nature) on the likely candidates that regulate the susceptibility to phase 2 VF. Indeed, there is reason to speculate that the identity and relative importance of different mediators and protectants differ from the situation during early ischaemia, owing to differences in the tissue milieu. For example, extracellular potassium levels rise to exceed 20 mM 60 min after the onset of ischaemia (Hill & Gettes, 1980). Such levels are incompatible with the persistence of sodium channel-dependent conduction, which is severely impaired after just 20 min of ischaemia (Kleber *et al*., 1986). Furthermore, the function of intrinsic cardiac nerves, whose importance as a source of protective neurotransmitters during early ischaemia

has been recently documented (Ferdinandy *et al*., 1997; Franco-Cereceda, 1988), is likely to be inhibited at these levels. Additionally, various cells of the inflammatory and necrotic processes, at relatively normal levels during early ischaemia, are found to accumulate during this period (Entman *et al*., 1993) with the potential to release a range of mediators (arrhythmogenic and protective) that are not encountered during early ischaemia.

Thus, the candidate mediators and protectants are likely to be different from those relevant to early ischaemia. Consequently, until a great deal more work is done, it is safe only to propose that some of the mediators and protectants relevant to early ischaemia may play a similar role during infarct evolution, but that other (unknown) substances are likely to be involved, and that further investigation is required.

Conclusion

Despite the ability of catecholamine infusion to restore an α- and β- drive to the heart, normalize heart rate and alter the ECG, the high susceptibility to phase 2 VF that occurs *in vivo* was not restored. This suggests additional factors (that are absent in Krebs-perfused hearts), are necessary for initiation of phase 2 VF. These factors include blood components such as neutrophils; they represent a focus for future studies.

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