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Effects of dofetilide on cardiovascular tissues from normo- and hypertensive rats

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Abstract

The aim was to test whether dofetilide has some potential for use in the treatment of heart failure. Dofetilide at $\leqslant 3\times10^{-5}$ M had no effect on the quiescent Wistar Kyoto (WKY) rat aorta, mesenteric and intralobar arteries, or the spontaneous contractions of the WKY rat portal vein. Dofetilide at 10^{-6} to 3×10^{-5} M relaxed the KCl-contracted aorta. Dofetilide at 10^{-9}–10^{-7} M augmented the force of contraction of left ventricle strips from 12- and 18-month-old WKY rats at 2 Hz. Spontaneously hypertensive rats (SHRs) at 12 and 17–21 months of age are models of cardiac hypertrophy and failure, respectively. The augmentation of force at 2 Hz with dofetilide was similar on 12- and 18-month-old WKY rats and 12-month-old SHRs but reduced on the 18-month-old SHR left ventricle. At a higher more physiological frequency, 4 Hz, the threshold concentration of dofetilide required to augment the force responses of 21-month-old SHR left ventricles was markedly increased and the maximum augmenting effect was decreased. Dofetilide at 10^{-7}–10^{-5} M reduced the rate of the 17-month-old WKY rat right atrium, and had a similar effect on age-matched SHR right atrium. In summary, dofetilide is a positive inotrope and negative chronotrope in the rat. However, as the positive inotropic effect is not observed with clinically relevant concentrations at a physiological rate in heart failure, dofetilide is unlikely to be useful as a positive inotrope in the treatment of heart failure.

Introduction

Potassium-channel blockers, which inhibit repolarization to prolong the cardiac action potential, are used in the treatment of cardiac arrhythmias (Roden 1996). Prolonging the action potential with non-selective potassium-channel blockers such as tetraethylammonium and 4-aminopyridine is associated with a positive inotropic effect on the spontaneously hypertensive rat (SHR) left ventricle strip (Nand & Doggrell 1999).

Dofetilide is a selective inhibitor of the fast component of the delayed outward rectifying potassium current (I_{kr}) (Jurkiewicz & Sanguinetti 1993). It has been the subject of clinical trials as a class III antiarrhythmic agent (Torp-Pedersen et al 1999; Kober et al 2000; Singh et al 2000) but has not been considered as a positive inotropic for the treatment of heart failure.

Potassium-channel blockade is not generally considered as a potential positive inotropic mechanism for the treatment of heart failure. There are a number of probable reasons for this, one being that the non-selective blockers have been shown to cause vasoconstriction (tetraethylammonium, 4-aminopyridine; Halliday et al 1995; Nand & Doggrell 1999) and this would be detrimental in failure. The effects of dofetilide on blood vessels have not been reported. Furthermore, despite

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predictions that prolonging the cardiac action potential with potassium-channel blockers will lead to a positive inotropic effect, there have been no studies quantifying the effects of dofetilide on force in the presence of cardiac disease.

Another possible reason for not considering potassium-channel blockers in heart failure is that they may have pro-arrhythmic effects in the absence or presence of β-adrenoceptor activation. Cardiac force is augmented by noradrenaline (norepinephrine) released from the sympathetic nervous system acting at β-adrenoceptors. Sympathetic nervous system activity is high in the failing heart to maintain cardiac output. It is possible that the combination of a potassium-channel blocker and a β-adrenoceptor agonist could be arrhythmogenic.

The overall aim of this study was to test whether dofetilide has some potential for use in the treatment of heart failure. Firstly, we characterized the effects of dofetilide on the isolated blood vessels from Wistar Kyoto normotensive (WKY) rats and spontaneously hypertensive rats (SHRs). Secondly, the positive inotropic effects of dofetilide were determined on left ventricles of WKY rats. Isolated cardiac muscles are used to define positive inotropic responses since loading conditions can be well controlled, in contrast to studies in the whole animal. These studies were performed in the absence and presence of isoprenaline to determine whether the potassium-channel blockers were pro-arrhythmogenic. Subsequently, we determined whether the positive inotropic effects of dofetilide were maintained in the presence of advanced cardiac hypertrophy and failure by studying the effects of the blockers on the left ventricle of 12- and 20-month-old SHRs. As hypertrophy in humans is usually associated with chronic hypertension, the SHR at 12 months is a realistic model of human hypertrophy (Doggrell & Brown 1998). Eighteen- to 21-month-old SHRs are in cardiac failure (Bing et al 1995). Finally, we tested the effects of dofetilide on the rate responses of the right atrium of WKY rats and SHRs.

Materials and Methods

Drugs

The drugs used in this study were atropine sulfate, guanethidine sulfate, (−)-isoprenaline bitartrate (Sigma Chemical Co.), ICI 118,551 (donated by ICI) and dofetilide (donated by Pfizer Ltd). All drugs, except dofetilide, were dissolved in distilled water. Dofetilide was dissolved at 1 M in 10^{-1} M hydrochloric acid.

Rats

Breeding pairs of WKY rats and Okamoto SHRs were purchased from the Animal Resources Centre, Perth, Western Australia and then colonies of these rats were established in the Animal Resource Unit, School of Medicine, The University of Auckland. Adult rats were housed three to a cage with free access to standard rat chow and water.

Beginning of the experiment

Rats were stunned and exsanguinated. The heart, aorta, portal vein, lungs and mesenteric bed were rapidly removed and placed in Krebs' solution that was saturated with 5% CO_2 in oxygen at 37°C. The free wall of the left ventricle was excised. Aorta and portal veins were cleared of surrounding tissue. Third-branch 1.6-mm lengths of mesenteric arteries and intralobar pulmonary arteries were dissected. All experiments were performed in the presence of a modified Krebs' solution (composition (mM): NaCl, 116; KCl, 5.4; $CaCl_2$, 2.5 (aorta, portal vein and left ventricle) or 1.5 (intralobar pulmonary and mesenteric arteries); $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 22.0; D-glucose, 11.2). In the ventricle experiments the Krebs' solution contained guanethidine at 10^{-5} M to prevent the release of noradrenaline (norepinephrine) from nerve endings and atropine at 10^{-6} M to block muscarinic receptors. Experiments were performed at 37°C.

Recording of contractions from the aorta

Endothelium-intact aortic rings from 9- to 12-month-old WKY rats were prepared and suspended under 1.5 g tension in 5-mL organ baths and equilibrated for 60 min, during which 250 mL Krebs' solution superfused the tissue. Contractility was measured isometrically with force-displacement transducers (Grass model FT03.C) and displayed on an eight channel Grass polygraph (model 79B). When testing the effect of dofetilide on the quiescent tissues, tissues were cumulatively challenged on a 5-min cycle. When testing the effects on the KCl-contracted tissue, the aortic rings were contracted by the addition of KCl at 15 mM to give a contraction of about 250 mg. If a contraction of 250 mg was not obtained with KCl at 15 mM, the concentration added was increased to 22.5 and then to 30 mM KCl. When the contractions to KCl were at a plateau, the aortae were challenged with dofetilide on a 5-min cycle. The attenuation to dofetilide was calculated as a percentage of the KCl contraction.

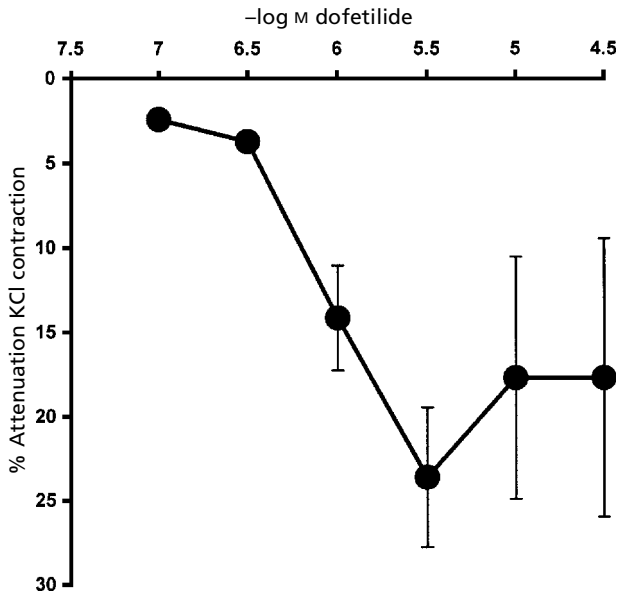

Figure 1 Effect of dofetilide on the KCl-contracted WKY rat aorta. The percentage relaxation is plotted against the negative logarithm of the molar concentration of dofetilide. Each value is the mean±s.e.m. from four aortae.

Recording of contractions from portal vein

Portal veins (about 12 mm) derived proximally to the liver of 14-week-old WKY rats were cleared of surrounding tissue and mounted longitudinally under 1 g tension in 5-mL organ baths containing Krebs' solution and allowed to equilibrate for 30 min. During this equilibration period the tissues were washed by the overflow of 300 mL Krebs' solution. Contractions were measured isometrically with Grass force transducers, displayed on a Grass polygraph. The wash was stopped and the contractions were allowed to stabilize, which took 20–30 min. Then a cumulative exposure to dofetilide on a 5-min cycle was made. The amplitudes of the final three contractions before the addition of each concentration of dofetilide were measured and averaged. Responses were calculated as a percentage change of the spontaneous contractile activity, and were corrected for changes in untreated tissues.

Recording of contractions from intralobar pulmonary and mesenteric arteries

The contractions of the intralobar pulmonary and mesenteric arteries of 14-week-old WKY rats and SHRs were recorded in the Mulvany myograph containing Krebs' solution at 37°C. After equilibration for 1 h the pulmonary arteries were set up under the equivalent of 15 mmHg and the mesenteric arteries from the WKY rats and SHRs under 60 and 80 mmHg, respectively. The tissues were equilibrated for 15 min before two challenges with K^+ physiological salt solution (K^+PSS (mM): NaCl, 37.9; KCl, 85.8; $CaCl_2$, 1.5; $MgCl_2$, 1.2; $NaHPO_4$, 1.2; $MgCl_2$, 1.2; $NaHCO_3$, 22.0; D-glucose 11.2). All the tissues challenged with K^+PSS responded. Vessels were then washed for 15 min before a cumulative challenge with dofetilide on a 3-min cycle or until a maximum response was obtained. Contractions were calculated as a percentage K^+PSS response.

Measurement of blood pressure and heart rate

14-week, 12- and 18-month old WKY rats or SHRs were weighed and then the tail cuff (systolic) blood pressure was measured using a tail plethysmograph (IITC Life Sci. Model 29). Thus, the rats were placed in Perspex holding cylinder and left in the dark for 30 min, during which time they routinely went to sleep. The occlusion cuff was placed around the tail, which had been warmed to 33°C under a reading light. The tail-cuff was inflated to 250 mmHg so that the arterial pulse displacements were no longer apparent. The pressure was gradually reduced until the pulse was observed on the chart recorder. The pulse point was recorded as the tail cuff pressure and the rate of the pulses as the in-vivo pulse (heart) rate. Three readings were taken per rat and these were usually very similar and were averaged.

Recording of the contractions from the electrically driven rat ventricle

The method used by us previously (Nand & Doggrell 1999) was followed. Five strips were prepared from the 12- or 18- to 21-month-old WKY rats or SHR left ventricle free wall. Four of the individual strips were mounted longitudinally between 2 platinum electrodes under 1 g tension in 5-mL organ baths in Krebs' solution being vigorously bubbled with 5% CO_2 in oxygen and allowed to equilibrate for 75 min. Stimulation at 2 or 4 Hz (5-ms duration) and at 2–3 threshold voltage, which was usually 30 V on the WKY rat and 60 V on the SHR, was commenced. After 6 min of stimulation, isoprenaline at 10^{-10} M was added with the contractions being recorded via a Grass polygraph onto a Grass Polyview. The cumulative addition of isoprenaline (10^{-9}, 10^{-8} M, etc.) occurred on a 3-min cycle until an isoprenaline maximal response was obtained. Stimulation was then stopped and three ventricle strips were treated with differing concentrations of dofetilide while the other strip remained untreated or was vehicle treated.

Table 1 Characteristics of 12- and 18-month-old WKY rats and SHRs.

	12-WKY	12-SHR	18-WKY	18-SHR
Age (days)	365±10	369±9	557±9	552±8
Weight (g)	454±12	463±13	456±13	379±14*
Heart rate (beats/min)	331±14	360±9*	280±10	390±12*
Tail-cuff blood pressure (mmHg)	132±8	188±9*	133±10	194±15*
Heart weights (% body wt $\times 10^{-2}$)				
Whole heart	24.41±0.25	30.10±0.40*	25.39±0.41	41.29±0.58*
Left ventricle	10.47±0.21	13.20±0.53*	10.94±0.34	17.66±0.66*
Septum	6.23±0.12	9.34±0.19*	6.14±0.53	10.89±0.23*
Right ventricle	5.83±0.19	5.81±0.16	6.38±0.43	10.14±0.32*
Atria	1.85±0.09	1.79±0.11	1.91±0.07	2.60±0.14*
Cardiac stimulation response at 2 Hz				
T_P (ms)	54.5±2.9	54.0±1.5	53.9±1.57	105.5±3.1*
Force (mg)	249±25	182±20*	253±36	179±32*
TR_{50} (ms)	53.9±2.1	62.3±1.5*	58.3±2.6	87.4±3.1*
TR_{90} (ms)	95.2±2.0	103.4±2.5*	97.1±2.3	114.6±2.7*

Each value is expressed as the mean±s.e.m. from 8 rats. *$P < 0.05$, vs age-matched WKY rats.

Strips were superfused with approximately 500 mL of Krebs' solution over 75 min before a second challenge to cardiac stimulation and isoprenaline. This procedure was repeated with the dofetilide-treated tissues receiving higher concentrations of the drug, and the untreated tissue remaining untreated or being vehicle treated.

The times to peak force (T_P) and to 50 and 90% relaxation (TR_{50} and TR_{90}) were measured in milliseconds. The force of contractions to cardiac stimulation was measured as milligrams tension. The force responses immediately before the second and third challenges to isoprenaline were calculated as a percentage of the force responses to stimulation before the first challenge with isoprenaline. If these force responses to electrical stimulation between treated and untreated tissues were significantly different, the percentage difference of the values from the individual treated tissues from the mean of the untreated tissues was calculated. The maximal combined responses to electrical stimulation and isoprenaline were measured as milligrams tension and as a percentage of the maximal response to the first challenge to stimulation and isoprenaline, and compared. The maximal combined responses were not significantly different between dofetilide-treated and untreated tissues in any of our experimental groups. Thus, the combined data were normalized.

Recording of rate from right atrium

The right atria from 17-month-old WKY rats and SHRs were mounted longitudinally under 0.5 g tension in 5-mL organ baths in guanethidine and atropine containing Krebs' solution. Atria were vigorously bubbled with 5% CO_2 in oxygen and allowed to equilibrate for 75 min during which about 250 mL of Krebs' superfused the tissue. The rate was recorded with a Grass tachygraph and recorded on a Grass polygraph. The cumulative addition of dofetilide (10^{-8}, 10^{-6} M, etc.) occurred on an 8-min cycle.

Tissue characteristics

The atria, the free wall of the left and right ventricles and septum were separated, blotted and weighed. Tissue weights were calculated as percentage body weight.

Statistical analysis

In all experiments mean values±s.e.m. were determined. Tests of significance between two groups were made by Student's paired or unpaired *t*-test, as appropriate, or, when more than two groups of data from several rats were involved, by analysis of variance followed by Student's unpaired *t*-test.

Results

Effect of dofetilide on blood vessels

Dofetilide at 10^{-9} to 3×10^{-5} M had no effect on the quiescent WKY rat aorta (n = 2, data not shown). Dofetilide at 10^{-7} M had no effect, and at 10^{-6} to 3×10^{-5}M relaxed the KCl-contracted aorta (Figure 1). Pre-

treatment with ICI 118,551 (a β_2-adrenoceptor selective antagonist) at 10^{-7} M had no effect on the relaxations of KCl-contracted aorta to dofetilide (n = 4, data not shown). Dofetilide at 10^{-9}–10^{-5} M had no effect on the amplitude of contractions of 8 portal veins (data not shown).

Dofetilide at 10^{-9}–10^{-3} M had no effect on quiescent mesenteric and intralobar pulmonary arteries of 14-week-old WKY rats and SHRs (n = 4, data not shown).

SHR model of hypertrophy and heart failure

The WKY and SHRs were age-matched at 12- and 18-months, and weight-matched at 12-months (Table 1). At 18-months the SHRs had lost weight, and were lighter than age-matched WKY rats (Table 1). Both 12- and 18-month-old SHRs had higher tail-cuff blood pressures and pulse rates than age-matched WKY rats (Table 1). The SHRs had greater heart weights than the WKY rats, and this represented a weight gain in the left ventricle and septum, but not in the right ventricle or atria at 12 months of age (Table 1). The weight gain in the SHR heart had extended to the right ventricle and atria at 18 months of age (Table 1).

Stimulation at 2 Hz caused contractions of the left ventricle strips that were increased by isoprenaline. With repeated stimulation of the left ventricle at 90-min intervals over 3 h, the peak responses to electrical stimulation were reduced and the magnitude of the responses to isoprenaline increased, but the time course of the contractions, T_P, TR_{50} and TR_{90}, was not altered (data not shown).

The times to peak (T_P) contraction values in response to electrical stimulation were similar from 12- and 18-month-old WKY and 12-month-old SHRs, but were almost doubled in the left ventricles of 18-month-old SHRs (Table 1). The magnitude of the contractions was less from the SHR than WKY rat left ventricles (Table 1). The times to relaxation (TR) values were similar on 12- and 18-month-old WKY, but lengthened on 12- and 18-month-old SHR left ventricles (Table 1).

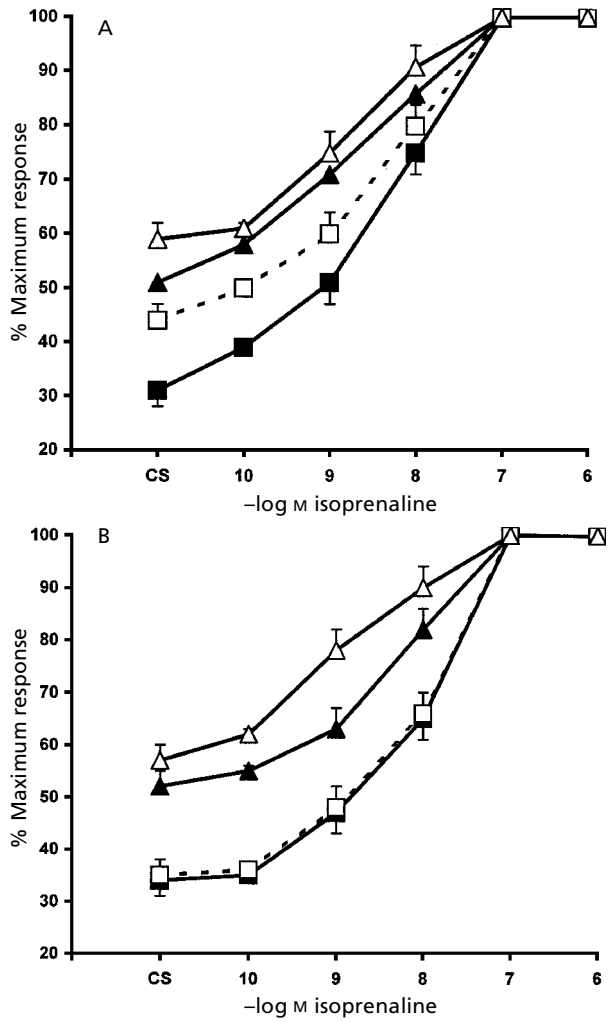

Figure 2 The effects of dofetilide on the contractions of left ventricles from 18-month-old WKY rats (A) and SHRs (B). Contractions from untreated ventricles (■) and from ventricles treated with dofetilide at 10^{-9} (□), 10^{-8} (▲) and 10^{-7} M (△). Contractions were calculated as a percentage of the maximum response to cardiac stimulation and isoprenaline, and plotted against cardiac stimulation alone (CS) and then the negative logarithm of the molar concentration of isoprenaline. Each value is the mean±s.e.m. from eight preparations.

Effect of dofetilide on left ventricle contractility at 2 Hz

Dofetilide at $\leqslant 3\times10^{-7}$ M had no effect on the T_P of the left ventricles of 12- and 18- month-old WKY or SHRs (data not shown). Dofetilide at 10^{-9} to 3×10^{-7} M augmented the peak amplitude of contractions in response to electrical stimulation. There was also an augmentation of the submaximal, but not maximal, responses in the presence of isoprenaline. The effect of dofetilide at 10^{-9}, 10^{-8} and 10^{-7} M on the force of 18-month-old WKY and SHR left ventricles is shown in Figure 2. These augmenting effects of dofetilide were similar on the left ventricles from 12- and 18-month old WKY rats and 12-month-old SHR but reduced on the 18-month-old SHRs (Table 2).

Dofetilide at 10^{-9} to 3×10^{-7} M prolonged the TR_{90} values by a greater percentage than the TR_{50} values (Table 2; $P < 0.05$ at each concentration). Dofetilide had similar effects on the ventricular relaxation of the 12- and 18-month old WKY rats and 12-month-old

Table 2 Effects of dofetilide on the force and length of contractions to electrical stimulation of left ventricles from 12- and 18-month-old WKY rats and SHRs.

Percentage augmentation of force				
Dofetilide (M)	**12-WKY**	**12-SHR**	**18-WKY**	**18-SHR**
10^{-9}	29±4	30±3	27±6	6±7*
3×10^{-9}	37±4	41±6	41±6	10±7*
10^{-8}	48±5	50±5	50±5	23±6*
3×10^{-8}	79±3	75±4	75±5	56±7*
10^{-7}	91±5	90±6	90±3	64±9*
3×10^{-7}	102±5	103±4	103±4	69±6*

Percentage lengthening of contraction				
	TR_{50}		**TR_{90}**	
Dofetilide (M)	18-WKY	18-SHR	18-WKY	18-SHR
10^{-9}	10.2±1.2	1.1±2.0*	20.3±3.1	2.1±2.9*
3×10^{-9}	12.4±1.6	1.7±1.8*	22.7±2.7	2.5±3.1*
10^{-8}	16.3±1.7	9.8±1.7*	27.3±1.7	5.9±1.7*
3×10^{-8}	20.9±1.8	10.3±1.5*	31.9±2.7	12.7±1.9*
10^{-7}	26.3±2.0	15.5±1.5*	34.9±2.8	17.7±2.9*
3×10^{-7}	30.9±2.1	20.9±2.7*	39.9±2.9	23.5±3.1*

Each value is expressed as the mean±s.e.m. from eight preparations. *$P < 0.05$, unpaired t-test, vs the same concentration of dofetilide on age-matched WKY rats.

SHRs but reduced effects on the 18-month-old SHRs (Table 2).

Effects of dofetilide on SHR left ventricle contractility at 4 Hz

These experiments were undertaken to determine whether the positive inotropic effects of dofetilide were present on SHR left ventricles at a higher frequency, 4 Hz compared with 2 Hz. In these experiments, 21-month-old SHRs (629 days±12, n = 8) with a right ventricle/body weight ratio of $8.84\pm0.84\times10^{-2}$ (8) were used.

Much higher concentrations of dofetilide were required to have a positive inotropic effect on the SHR left ventricle stimulated at 4 Hz than at 2 Hz. Thus the concentrations that had an effect, including a maximum effect (10^{-9} to 3×10^{-7} M) at 2 Hz, had no effect on the force of contractions at 4 Hz (data not shown). Dofetilide at 10^{-6} and 3×10^{-6} M augmented the magnitude of the response to electrical stimulation at 4 Hz by 28±10% (4) and 46±7% (4), respectively, and this produced an augmentation of the submaximal, but not the maximal, responses in the presence of isoprenaline (Figure 3). Dofetilide at 10^{-5} M had no effect. On the SHR left ventricle, the 46±7% (4) maximum augmentation with dofetilide at 4 Hz was considerably less than the 69±6% (8) at 2 Hz.

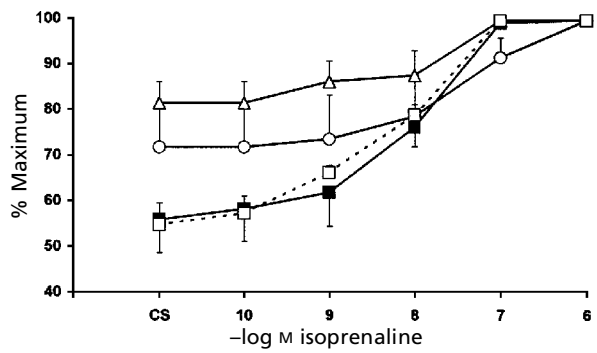

Figure 3 Effect of dofetilide on the contractions of left ventricles from 21-month-old SHRs, driven at 4 Hz. Contractions from untreated ventricles (□) and from ventricles treated with dofetilide at 10^{-6} (○), 3×10^{-6} (△) and 10^{-5} M (■). Contractions were calculated as a percentage of the maximum response to cardiac stimulation and isoprenaline, and plotted against cardiac stimulation alone (CS) and then the negative logarithm of the molar concentration of isoprenaline. Each value is the mean±s.e.m. from eight preparations.

Effects of dofetilide on right atrium rate

In these experiments, 17-month-old WKY rats (504±10 days (4)) and SHRs (520±10 days (4)) were used.

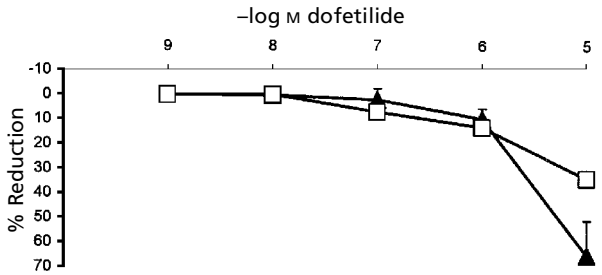

Figure 4 Effects of dofetilide on rate of the right atrium of 17-month-old WKY rats (▲) and SHRs (□). The percentage reduction from basal rate was plotted against the negative logarithm of the molar concentration of dofetilide. Each value is the mean±s.e.m. from eight preparations.

Seventeen-month-old SHRs have a weight gain in the right ventricle but not in the atria (data not shown).

The in-vitro basal rate in right atrium from WKY rats was 206±26 (4) beats min^{-1}, and a similar rate was observed on the SHR right atrium. Dofetilide at 10^{-8} M had no effect and at 10^{-7}–10^{-5} M reduced the rate of the WKY rat and SHR right atrium to a similar extent (Figure 4).

Discussion

Non-selective potassium-channel blockers cause vasoconstriction, which is an unwanted effect in heart failure. This study shows that dofetilide did not contract large or fine rat blood vessels. High concentrations of dofetilide, $\geqslant 10^{-6}$ M, relaxed the KCl-contracted aorta. To our knowledge, dofetilide has not previously been reported to relax blood vessels. In clinical studies, the plasma concentrations of dofetilide are 5×10^{-9} to 3×10^{-8} M (Sedgewick et al 1992; Falk et al 1997; Norgaard et al 1999). Thus the vasodilator effect of dofetilide probably does not occur with clinical use.

Dofetilide attenuates the KCl-induced contractions of the rat aorta by an ICI 118,551 resistant mechanism and thus β_2-adrenoceptors are not involved in this attenuation. The objective of the vascular part of this study was to ascertain whether dofetilide induced vasoconstriction. The vasodilatory effect observed with dofetilide was novel, and the mechanism remains unknown.

Positive inotropic effects have been reported with dofetilide on the guinea-pig papillary muscle (Tande et al 1990). Given that dofetilide has been shown to have a positive inotropic effect in this study on the rat left ventricle, it may be worthwhile to test the effects on human isolated heart force. One of the main aims of this study was to determine whether the positive inotropic effects of dofetilide were maintained in the presence of advanced cardiac hypertrophy and failure, and we did this by studying the effects on the SHR left ventricles. As hypertrophy and then failure in humans is usually associated with chronic hypertension, the 12- and 18-month-old SHRs are a realistic model of human hypertrophy and failure, respectively (Doggrell & Brown 1998). We showed that at 12 and 18 months of age, SHRs have left ventricular hypertrophy, impaired contractility of the left ventricle, and increased in-vivo pulse rates. The increased pulse rate is probably due to reflex activation of the sympathetic nervous system to the heart to maintain cardiac output as the heart fails, and is much greater in SHRs at 18 than at 12 months.

The SHR as a model of heart failure has been characterized by Bing et al (1995). Of many markers tested, the most consistent marker of the SHR in failure was right ventricular hypertrophy (Bing et al 1995). All of our SHRs at 17–21 months had right ventricular hypertrophy. Bing et al (1995) have divided their 18- to 24-month-old SHRs into 2 groups SHR-F (failing) which have right ventricular hypertrophy and SHR-NF (non-failing) which do not have right ventricular hypertrophy. Our 17- to 21-month-old SHRs are a homogeneous SHR-F group as they all had right ventricular hypertrophy.

It seems likely that our 18-month-old SHRs have a mixture of diastolic and systolic dysfunction in their heart failure. The prolonged relaxation of the left ventricle is a marker of diastolic dysfunction, and the prolongation of time to peak contraction is indicative of systolic dysfunction. I_{to}, but not I_K, potassium current is reduced in SHR hypertrophy (Cerbai et al 1994; Yokoshiki et al 1997). The positive inotropic effects of blockers of I_K are similar on the left ventricles of 12-month-old SHRs and WKY rats (D-sotalol, Doggrell & Nand 1998; bretylium, Nand & Doggrell 1999; clofilium, Nand & Doggrell 2000a; azimilide, Nand & Doggrell 2000b). This provides supportive evidence that the function of the I_K is not altered in the hypertrophied left ventricles of 12-month-old SHRs. This study demonstrates that the positive inotropic effects of dofetilide are also not altered in hypertrophied left ventricles of 12-month-old SHRs.

In cardiomyocytes from 18-month-old SHR heart failure, the I_{to} remained reduced with no effect on I_K (Cerbai et al 1994). In this study we show that the positive inotropic effects of dofetilide were reduced on the failing left ventricles of 18-month-old SHRs. It seems unlikely that these reduced effects of dofetilide are due to reduced I_{to}. This is because, at 12 months, I_{to} is

reduced but the positive inotropic effects of dofetilide are not. Also, it has been shown that dofetilide does not inhibit I_{to} (Carmeliet 1992). Further ion-channel studies, and studies with selective inhibitors of the fast and slow components are needed to clarify whether the I_K is altered as heart failure progresses.

The reduction in I_{to} in the hypertrophied heart is associated with altered intracellular Ca^{2+} handling, and may predispose to cardiac arrhythmia (Tomaselli & Marbán 1999). Increased sympathetic activity in heart failure may also be pro-arrhythmic. In our contractility studies, we did not observe arrhythmias in the absence or presence of dofetilide, and in the absence or presence of isoprenaline (data not shown). Dofetilide also does not cause excessive lengthening of WKY rat left ventricular action potentials (Nand & Doggrell 1997). This suggests the pro-arrhythmogenic potential of dofetilide may be low.

Prolongation of relaxation is an undesirable property in a drug for heart failure. Ideally drugs used as positive inotropes in heart failure should increase force with little or no effect on relaxation. The relaxation of SHR left ventricles was prolonged, and dofetilide further prolonged relaxation.

Reverse use-dependency is reduced effects when the stimulus frequency is increased (i.e., the action potential is prolonged to a greater extent at lower than at faster frequencies) (Hondeghem & Snyders 1990). Dofetilide shows marked reverse use-dependence (Knilans et al 1991; Bosch et al 1998). It is possible that inotropic effects may be reduced at higher more physiological frequencies. In the rat, 4 Hz is a physiological frequency. This study shows that higher concentrations of dofetilide are required to have a positive inotropic effect on the SHR left ventricle in failure at 4 than at 2 Hz. This alteration in effective concentrations has clinical implications. The effects with dofetilide at 4 Hz do not occur with clinically relevant concentrations (Sedgewick et al 1992; Falk et al 1997; Norgaard et al 1999). Frequency also has an effect on the maximal inotropic effect observed with a decrease with dofetilide at 4 Hz compared with 2 Hz.

In summary, dofetilide is not a vasoconstrictor on isolated rat blood vessels. Dofetilide has positive inotropic effects on the rat left ventricles driven at 2 Hz that are fully or partially maintained in hypertrophy and failure. At a physiologic frequency, 4 Hz, the threshold concentration of dofetilide required to augment the force responses of SHR left ventricles was markedly increased, and the maximum augmenting effect was decreased. Clinical concentrations of dofetilide are 5×10^{-9} to 3×10^{-8} M (Sedgewick et al 1992; Falk et al 1997; Norgaard et al 1999). Thus the effects of dofetilide on the SHR left ventricles observed in this study are with concentrations above those presently used in the clinic. In conclusion, dofetilide is unlikely to be useful as a positive inotrope in the treatment of heart failure.

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