J. Pharm. Pharmacol. 1999, 51: 631–640
Received August 14, 1998
Accepted December 9, 1998

Effects of Tetraethylammonium, 4-Aminopyridine and Bretylium on Cardiovascular Tissues from Normo- and Hypertensive Rats

VINITA NAND AND SHEILA A. DOGGRELL

Cardiovascular Pharmacology Group, Faculty of Medicine and Health Science, The University of Auckland, Private Bag 92109, Auckland, New Zealand

Abstract

Our objective was to test whether potassium-channel blockade is a potential positive inotropic mechanism for heart failure. Thus we studied the effects of tetraethylammonium, 4-aminopyridine and bretylium on left ventricular action potentials, left ventricular contractility in the absence and presence of hypertrophy, and on isolated blood vessels from Wistar Kyoto normotensive rats (WKY) and spontaneously hypertensive rats (SHRs).

Tetraethylammonium at 10^{-3}–10^{-2} M, 4-aminopyridine at 10^{-4}–10^{-3} M and bretylium at 10^{-6}–10^{-4} M prolonged the action potentials of the WKY left ventricular strip. Similar concentrations of tetraethylammonium, 4-aminopyridine and bretylium augmented the peak force, prolonged the contractions, and did not cause arrhythmias in the absence or presence of isoprenaline on left ventricular strips from 12-month-old WKY. The 12-month-old SHR has hypertrophy of the left ventricle with reduced contractility and prolongation of relaxation. The effects of tetraethylammonium and bretylium were similar on WKY and SHR, whereas the effects of 4-aminopyridine were reduced on SHR left ventricular contractility, which suggests that the function of the transient outward-blocking potassium channel may be impaired in hypertrophy. Bretylium at $\leq 10^{-4}$ M had no effect on the portal vein, intralobar or mesenteric arteries. Tetraethylammonium and 4-aminopyridine at $\geq 10^{-5}$ M increased the duration or amplitude, or both, of the portal vein contractions. Tetraethylammonium at $\geq 10^{-2}$ M and 4-aminopyridine at $\geq 3 \times 10^{-4}$ M contracted the mesenteric artery, and 4-aminopyridine also contracted the intralobar pulmonary artery.

In summary, we have demonstrated that the action potential prolonging effects of potassium-channel blockade is associated with a positive inotropic effect on the rat left ventricle. The non-specific blockers, tetraethylammonium and 4-aminopyridine, do not have potential as positive inotropes in heart failure because of their widespread effects, including vasoconstriction. The potential of bretylium and some of the newer selective potassium-channel blockers as positive inotropes requires further evaluation.

Although positive inotropes provide a cornerstone in the treatment of heart failure, the inadequacy of the currently used agents is well recognised and has provided the impetus for the continuing development of new approaches (Dorigo et al 1994). Potassium-channel blockers, which inhibit repolarization to prolong the cardiac action potential, are commonly used in the treatment of cardiac arrhythmias (Singh & Ahmed 1994; Colatsky 1995). Prolongation of the action potential is often associated with a positive inotropic effect, but potassium-channel blockade is not generally considered as a potential positive inotropic mechanism for the treatment of heart failure. There are a number of possible reasons for this. Thus appropriate studies have not been undertaken to ascertain whether potassium-channel blockade has positive inotropic effects that are maintained in the presence of cardiac disease. Our present study illustrates that potassium-channel blockers are positive inotropes.

One 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 aug-

Correspondence: S. A. Doggrell, Cardiovascular Pharmacology Group, Faculty of Medicine and Health Science, The University of Auckland, Private Bag 92109, Auckland, New Zealand.

E-Mail: s.doggrell@auckland.ac.nz

Preliminary reports of the cardiac and vascular data have been presented to the Australasian Section of the International Society for Heart Research and the Australasian Society for Clinical and Experimental Pharmacology and Toxicology, respectively.

mented by noradrenaline released from the sympathetic nervous system acting at β-adrenoceptors. Sympathetic nervous system activity is high in the failing heart to maintain cardiac output (Katz 1991). It is possible that combination of a potassium-channel blocker and a β-adrenoceptor agonist could be arrhythmogenic. A final possible reason for not considering potassium-channel blockade in heart failure is that they may cause vasoconstriction that would be detrimental in failure.

The overall aim of this study was to test whether potassium-channel blockade is a potential positive inotropic mechanism for treatment of heart failure. Firstly, we determined the effects of blocking potassium channels with tetraethylammonium, 4-aminopyridine or bretylium on the action potentials of left ventricles isolated from Wistar Kyoto normotensive rats (WKY), and showed a prolongation of the potentials. Tetraethylammonium is a non-selective blocker, 4-aminopyridine acts predominantly on the voltage-dependent outward rectifying potassium channel and bretylium is a selective blocker of the delayed outward rectifying potassium channel (Doggrell et al 1998). Secondly, we determined whether potassium-channel blockade was associated with positive inotropism. Isolated cardiac muscles were used to define positive inotropic responses since loading conditions could be well controlled, in contrast to studies using the whole animal. Thus we determined the effects of the 3 potassium-channel blockers on the force responses of WKY left ventricles, and showed that these blockers were positive inotropes. These studies were performed in the absence and presence of isoprenaline to determine whether the potassium-channel blockers were pro-arrhythmogenic.

Thirdly we determined whether the positive inotropic effects were maintained in the presence of advanced cardiac hypertrophy by studying the effects of the blockers on the left ventricle of 12-month-old spontaneously hypertensive rats (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). Finally we determined whether potassium-channel blockade produced vasoconstriction by studying the effects of the blockers on the isolated portal vein, intralobar pulmonary and mesenteric arteries.

Materials and Methods

Rats

Breeding pairs of WKY and Okamoto SHRs were purchased from the Animal Resources Centre, Perth, Western Australia and 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.

Measurement of blood pressure and heart rate

Fourteen-week- and 12-month-old WKY or SHRs were weighed and the tail cuff (systolic) blood pressure was measured using a tail plethysmograph (IITC Life Sci. Model 29). To do this the rats were placed in a 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.

Preparation of tissues

Rats were stunned and exsanguinated. The heart, portal vein, lungs and mesenteric bed were rapidly removed and placed in Krebs solution that was saturated with 5% CO_2 in oxygen and maintained at 37°C, and the free wall of the left ventricle was excised. Fourth generation 1·6-mm lengths of mesenteric arteries were dissected from the mesenteric bed associated with a 6-cm section of intestine, 12–18 cm from the caecum. Third generation intralobar pulmonary arteries were dissected out from the left lung. All experiments were performed in the presence of a modified Krebs solution, composition (mM): NaCl, 116; KCl, 5·4; $CaCl_2$, 2·5 (left ventricle and portal vein) 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 contained guanethidine at 10^{-5} M to prevent the release of noradrenaline from nerve endings and atropine at 10^{-6} M to block muscarinic receptors at 37°C. 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.

Recording of the action potentials from the electrically driven rat ventricle

The method used by us previously (Nand et al 1997) was followed. A strip of left ventricle that represented about a fifth of the ventricle was pinned endocardial-side uppermost to the silica gel base of a recording chamber between two platinum-stimulating electrodes. The tissues were superfused at approximately 2 mL min^{-1} with Krebs solution that had been vigorously bubbled with 5% CO_2 in oxygen before entering the recording chamber. Tissues were equilibrated for 60 min before the tip of a 3 M KCl-filled microelectrode was inserted into a cell close to the surface of the tissue. The tissues were then stimulated at 2 Hz, 5-ms duration and 30 V, and the resulting action potentials were amplified via a microelectrode preamplifier and displayed on an oscilloscope. After 3 min of stimulation, three action potentials were recorded from a single cell before the stimulator was turned off and tetraethylammonium, 4-aminopyridine or bretylium was added to the Krebs solution perfusing the tissue. After a 60-min equilibration, a further period of stimulation and recording of three action potentials was undertaken from the same cell. This procedure was repeated with higher concentrations of tetraethylammonium, 4-aminopyridine, bretylium or vehicle. The diastolic potential, peak amplitude and action potential duration at 50 and 90% repolarization (APD50 and APD90) were measured.

Recording of the contractions from the electrically driven rat ventricle

The method used by us previously (Nand et al 1997) was followed. Five strips were prepared from the left ventricle free wall. Four of the individual strips were mounted longitudinally between two 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 Hz (5 ms duration, 30 V) was commenced and after 3–6 min, 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 tetraethylammonium, 4-aminopyridine or bretylium while the other strip remained untreated or was vehicle treated. Strips were superfused with approximately 500 mL Krebs solution over 75 min before a second challenge to cardiac stimulation and isoprenaline. This procedure was repeated with the tetraethylammonium, 4-aminopyridine or bretylium 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 (TR50 and TR90) were measured in ms. The force of contractions to cardiac stimulation was measured as mg tension. The force responses immediately before the second and third challenges to isoprenaline were calculated as a percentage of the force responses to stimulation immediately before the first isoprenaline challenge. If these force responses to cardiac 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 cardiac stimulation and isoprenaline were measured as mg 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 tetraethylammonium, 4-aminopyridine and bretylium-treated and untreated tissues in any of our experimental groups, thus, the combined data were normalised.

Recording of contractions from portal vein

Portal veins (about 12 mm) derived proximally to the liver of 14-week-old WKY 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 and the area of the contractions was determined with a Grass integrator. The wash was stopped and the contractions were allowed to stabilize, which took 20–30 min. A cumulative exposure to tetraethylammonium, 4-aminopyridine or bretylium on a 5-min cycle was made. The amplitudes of the final three contractions and the integration of the final 3 min before the addition of each concentration of tetraethylammonium, 4-aminopyridine or bretylium, were measured, and the contractions were 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 artery ring preparations of 14-week-old WKY and SHRs were recorded in the Mulvany myograph containing Krebs solution at 37°C, bubbled with 95% O_2/5% CO_2. Rings were mounted on stainless steel wires (0·04 mm diameter) and equilibrated for 1 h. The pulmonary arteries were individually normalised to a resting force that corresponded to a transmural pressure of 15 mmHg and the mesenteric arteries from the WKY and SHRs under 60 and 80 mmHg, respectively, as described by Mulvany & Halpern (1976). The resting forces approximate to the in-vivo pressure in normo- and hypertensive rats. The tissues were equilibrated for 15 min before two challenges with 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). The tissues were then washed for 15 min before a cumulative challenge to tetraethylammonium, 4-aminopyridine or bretylium on a 3-min cycle or until a maximum response was obtained. Contractions were calculated as a percentage of the K^+PSS response.

Tissue characteristics

At the end of the contractility experiment the strips of ventricle were removed from the organ baths and the lengths, diameter and wall radius measured before the tissues were blotted and weighed. The heart and the separated left ventricle were blotted and weighed.

Drugs

The drugs used in this study were all dissolved in distilled water and were 4-aminopyridine, atropine sulphate, bretylium tosylate, guanethidine sulphate, (−)isoprenaline bitartrate and tetraethylammonium chloride (Sigma Chemical Co.).

Results

Left ventricle

Action potentials. Direct cardiac stimulation at 2 Hz (5 ms, 30 V) of the left ventricles from 14-week-old WKY caused action potentials that were stable during repeated stimulations over 3 h (n = 5 for each group, data not shown).

Tetraethylammonium at 10^{-2} M, 4-aminopyridine at 10^{-2} M and bretylium at 10^{-4} M had no effect on the diastolic membrane potential of −82 mV (data not shown). Tetraethylammonium at 10^{-4} M had no effect on the action potentials, at 10^{-3} M had no effect on the amplitude but prolonged the potentials and at 10^{-2} M increased the amplitude and duration (Table 1). 4-Aminopyridine at 10^{-4} and 10^{-3} M increased the amplitude and prolonged the action potentials from the left ventricle. In the presence of 4-aminopyridine at 10^{-2} M, action potentials could not be elicited at 2 Hz. Bretylium at 10^{-6} M had no effect on the amplitude but increased the duration, and at 10^{-5} and 10^{-4} M increased the amplitude and duration of the left ventricular action potentials. Tetraethylammonium, 4-aminopyridine at 10^{-4}–10^{-3} M and bretylium at 10^{-5}–10^{-4} M caused a greater % increase in the action potential duration at 50 than 90% repolarization.

Contractions. The WKY and SHRs were age-matched at 12 months, and were weight-matched (Table 2). The SHRs had higher tail cuff blood

Table 1. Effects of tetraethylammonium, 4-aminopyridine and bretylium on left ventricle action potentials in 14-week-old normotensive rats.

	Amplitude (mV)	APD50 (ms)	% Increase	APD90 (ms)	% Increase
Untreated	95·7 ± 0·9	3·74 ± 0·18		6·14 ± 0·14	
TEA (10^{-4} M)	95·6 ± 1·3	3·80 ± 0·22		6·20 ± 0·16	
TEA (10^{-3} M)	95·6 ± 0·6	6·89 ± 0·21*	86 ± 9	9·20 ± 0·27*	51 ± 6†
TEA (10^{-2} M)	111·5 ± 0·6*	8·53 ± 0·28*	130 ± 5	12·03 ± 0·18*	96 ± 4†
Untreated	95·6 ± 0·6	3·70 ± 0·20		6·10 ± 0·16	
4-AP (10^{-4} M)	112·9 ± 1·7*	5·78 ± 0·13*	58 ± 6	8·99 ± 0·49*	48 ± 9
4-AP (10^{-3} M)	137·7 ± 0·5*	12·01 ± 0·50*	222 ± 5	16·19 ± 0·26*	166 ± 8†
Untreated	95·8 ± 1·2	3·80 ± 0·4		6·30 ± 0·20	
Bretylium (10^{-6} M)	96·2 ± 0·7	7·02 ± 0·23*	82 ± 6	11·65 ± 0·27*	84 ± 4
Bretylium (10^{-5} M)	103·8 ± 0·7*	15·39 ± 0·36*	299 ± 7	20·36 ± 0·18*	221 ± 3†
Bretylium (10^{-4} M)	108·6 ± 1·2*	21·65 ± 0·33*	462 ± 8	22·31 ± 0·20*	252 ± 3†

TEA = tetraethylammonium. 4-AP = 4-aminopyridine. Each value is the mean ± s.e.m. Number of rats in each group = 4; *$P < 0·05$ compared with untreated animals. †$P < 0·05$ compared with APD50 increase.

Table 2. WKY and SHR characteristics.

	WKY	SHR
Age (days)	368 ± 2	364 ± 1
Weight (g)	425 ± 3	421 ± 3
Heart rate (beats min^{-1})	304 ± 4	350 ± 3*
Tail cuff b.p. (mmHg)	131 ± 2	192 ± 1*
Heart weight (mg)	1080 ± 15	1326 ± 31*
Left ventricle (mg)	467 ± 12	625 ± 7*
Left ventricle strips		
Length (mm)	15·1 ± 0·2	16·4 ± 0·2*
Diameter (mm)	4·8 ± 0·2	5·4 ± 0·1*
Wall radius (mm)	2·0 ± 0·1	2·3 ± 0·1

Each value is the mean ± s.e.m. from 24 rats. *$P < 0.05$, compared with age-matched WKY.

pressures and pulse rates than the WKY (Table 2). The SHR had greater heart weights than the WKY and this represented a weight gain in the left ventricle and septum but not in the right ventricle or atria. The SHRs left ventricle strips were longer and had a greater wall radius, but had a similar diameter to those of the WKY.

Stimulation at 2 Hz (5 ms, 30 V) caused contractions of the left ventricle strips which were increased by isoprenaline. With repeated stimulation of the left ventricle at 90-min intervals over 3 h, the peak cardiac stimulation responses were reduced and the magnitude of the responses to isoprenaline increased (data not shown). However the time-course of the contractions, time to peak (T_P), time to 50 and 90% relaxation (TR50 and TR90), was not altered with repeated stimulation (data not shown).The T_P values for the cardiac stimulation responses were similar from 12-month-old SHR and WKY left ventricles (Table 3). The magnitude of the contractions was less and the TR values were longer in the SHR than WKY left ventricles (Table 3).

Table 3. Contraction parameters from 12-month old WKY and SHR left ventricles.

	WKY	SHR
Cardiac stimulation response		
T_P (ms)	55·6 ± 1·0	55·8 ± 1·0
Force (mg)	221 ± 22	161 ± 13*
TR50 (ms)	55·8 ± 1·0	61·0 ± 0·8*
TR90 (ms)	101·8 ± 2·2	109·9 ± 2·7*
Maximum response in presence of isoprenaline		
T_P (ms)	55·2 ± 0·10	55·4 ± 0·11
Force (mg)	441 ± 36	320 ± 22*
TR50 (ms)	51·8 ± 1·5	57·2 ± 1·0*
TR90 (ms)	96·2 ± 2·3	104·4 ± 2·6*

Each value is the mean ± s.e.m. Number of rats in each group = 24, *$P < 0.05$, compared with WKY.

In the presence of isoprenaline at 10^{-7} M, which exerted a maximum force response, the T_P values were unchanged. The left ventricular force was doubled by isoprenaline at 10^{-7} M on the WKY and SHR, but remained less on the SHR than WKY (Table 3). Isoprenaline shortened the relaxation by similar amounts on the WKY and SHR ventricles, 0·39 ms at the TR50 and 0·55 ms at the TR90.

Tetraethylammonium at 10^{-4} M had no effect on the contractions, and $\leq 10^{-2}$ M had no effect on the T_P of the contractions of left ventricles of 12-month-old WKY or SHRs, (data not shown). Tetraethylammonium at 3×10^{-4}–10^{-2} M augmented the peak force of contractions to cardiac stimulation and this produced an augmentation of the submaximal, but not maximal, responses in the presence of isoprenaline; these augmenting effects were similar on the WKY and SHR (Figures 1 and 2). Tetraethylammonium prolonged the TR50 values by a significantly greater amount than the TR90 values. Thus on the WKY, tetra-

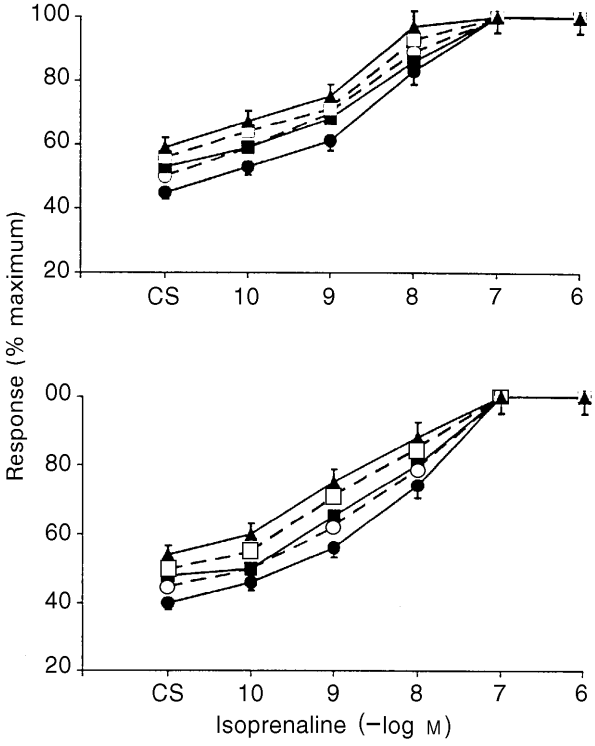

Figure 1. The effects of tetraethylammonium on the contractions of left ventricles from 12-month-old WKY (top) and SHRs (bottom). Contractions from untreated ventricles (●) and ventricles treated with tetraethylammonium at 10^{-4} (○), 3×10^{-4} (■), 10^{-3} (□) and 3×10^{-3} M (▲). Contractions were 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 (the s.e.m. are mostly contained within the symbol size).

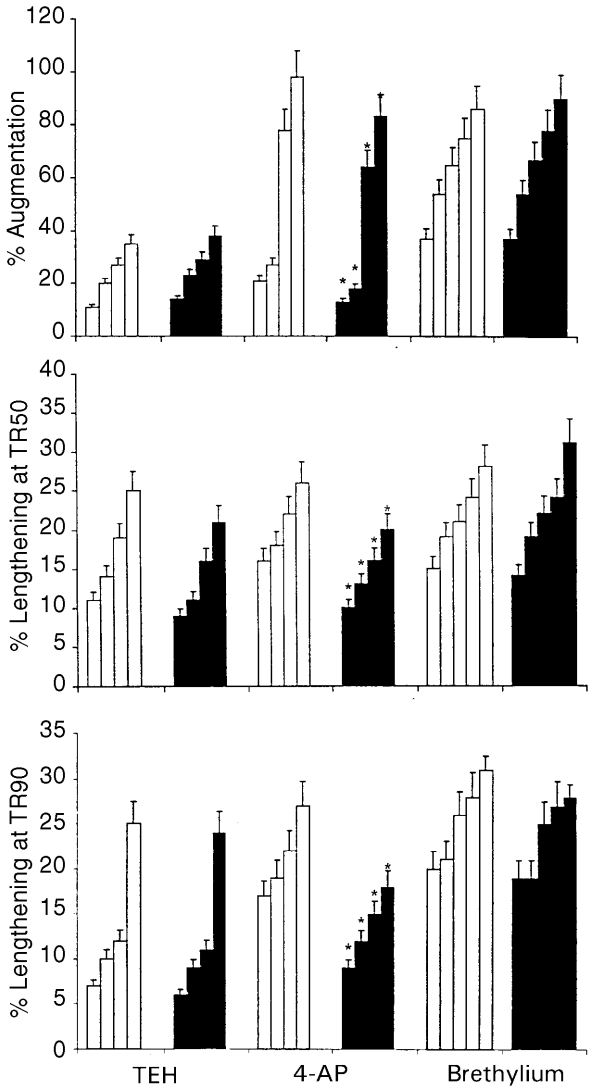

Figure 2. The effects of tetraethylammonium(TEA), 4-aminopyridine (4-AP) and bretylium on the contractions of left ventricles from 12-month-old WKY (□) and SHRs (■) as % augmentation of force (top), and % lengthening of TR50 (middle) and TR90 (bottom). Values, left to right: effects of tetraethylammonium at 10^{-4}, 3×10^{-4}, 10^{-3} and 3×10^{-3} M; 4-aminopyridine at 10^{-4}, 3×10^{-4}, 10^{-3} and 3×10^{-3} M, and bretylium at 10^{-6}, 3×10^{-6}, 10^{-5}, 3×10^{-5} and 10^{-4} M. Each value is the mean ± s.e.m. from eight preparations.

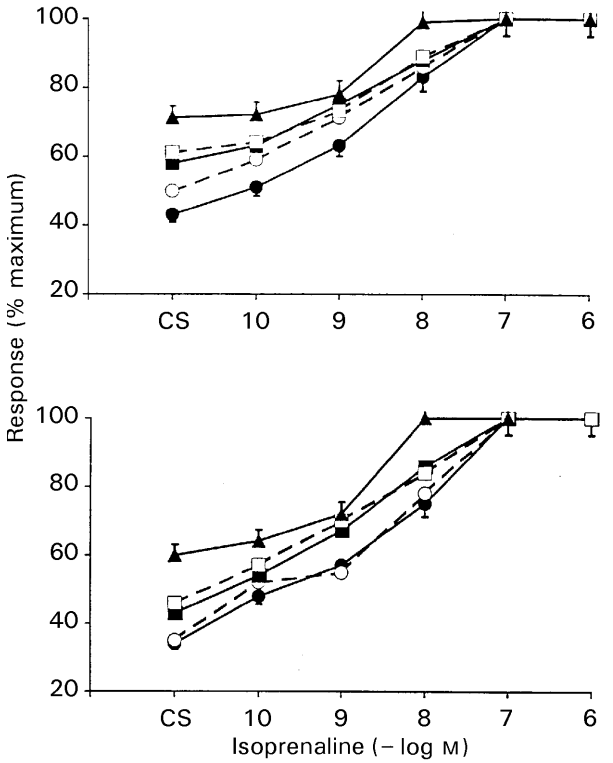

Figure 3. The effects of 4-aminopyridine on the contractions of left ventricles from 12-month-old WKY (top) and SHRs (bottom). Contractions from untreated ventricles (●) and ventricles treated with 4-aminopyridine at 10^{-4} (○), 3×10^{-4} (■), 10^{-3} (□) and 3×10^{-3} M (▲). Contractions were calculated 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 (the s.e.m. are mostly contained within the symbol size).

ethylammonium at 3×10^{-4}, 10^{-3}, 3×10^{-3} and 10^{-2} M prolonged the TR50 values by 11, 14, 19 and 25% and the TR90 values by 7, 10, 12 and 25%, respectively. Tetraethylammonium prolonged the relaxation times of the SHR left ventricular contractions by a similar degree to that of WKY (Figure 2).

4-Aminopyridine at 3×10^{-5} M had no effect on the contractions, and $\leq 3 \times 10^{-3}$ M had no effect on the T_P of the contractions of left ventricles of 12-month-old WKY or SHRs, (data not shown). 4-Aminopyridine at 10^{-4}–3×10^{-3} M augmented the peak force of contractions to cardiac stimulation and this produced an augmentation of the submaximal, but not maximal, responses in the presence of isoprenaline (Figure 3). This augmentation of force was lesser on the SHR than on the WKY left ventricle (Figure 2). 4-Aminopyridine at 10^{-4}, 3×10^{-4}, 10^{-3} and 3×10^{-3} M prolonged the TR50 and TR90 values to a similar extent, but the prolongation was less on the SHR than WKY left ventricle (Figure 2). 4-Aminopyridine at 10^{-2} M abolished contractions.

Bretylium at 3×10^{-7} M had no effect on the contractions, and at $\leq 10^{-4}$ M had no effect on the T_P of the left ventricles of 12-month-old WKY or SHRs (data not shown). Bretylium at 10^{-6}–10^{-4} M augmented the peak contractions to cardiac stimulation and this produced an augmentation of the submaximal (but not maximal) responses in the presence of isoprenaline, and these augmenting effects were similar on the WKY and SHR (Figure 4).

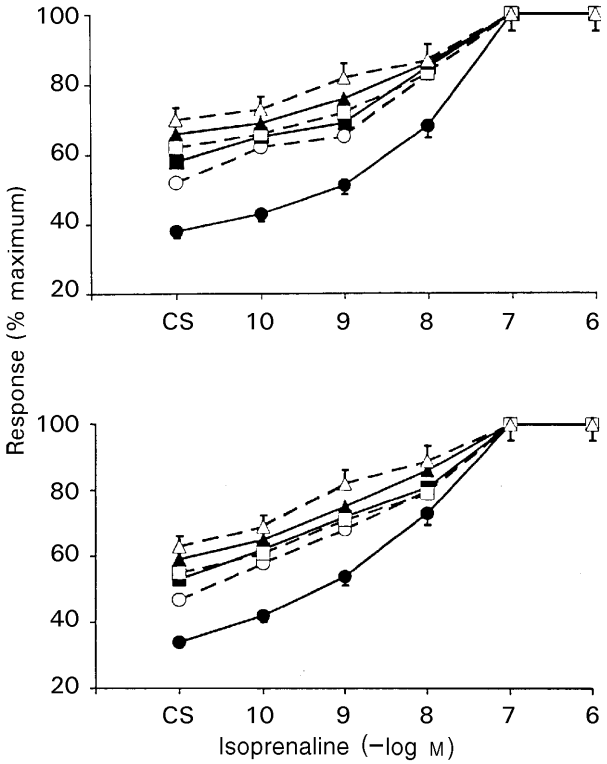

Figure 4. The effects of bretylium on the contractions of left ventricles from 12-month-old WKY (top) and SHRs (bottom). Contractions from untreated ventricles (●) and ventricles treated with bretylium at 10^{-6} (○), 3×10^{-6} (■), 10^{-5} (□), 3×10^{-5} (△) and 10^{-4} (△). Contractions were calculated as a % 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 (the s.e.m. are mostly contained within the symbol size).

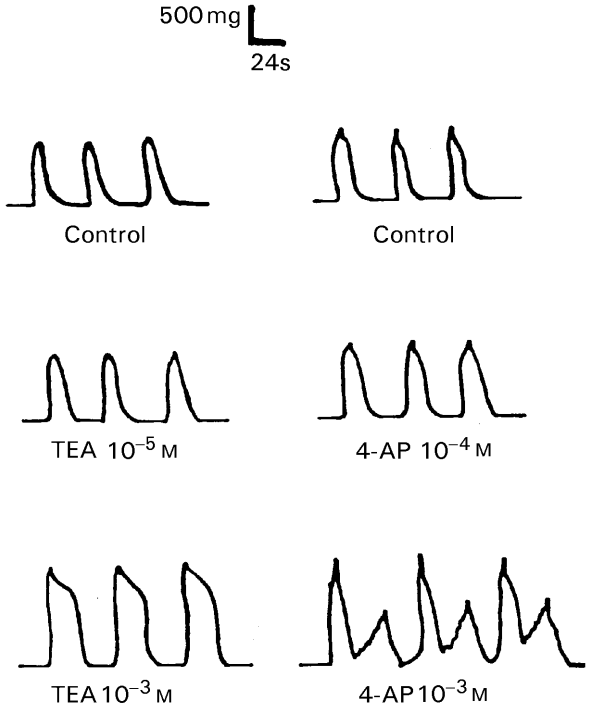

Figure 5. The effects of tetraethylammonium (TEA; left) and 4-aminopyridine (4-AP; right) on the portal veins of 14-week-old WKY.

Bretylium at 10^{-6}–10^{-4} M prolonged the TR90 values by a greater percentage than the TR90 values, and had similar effects on the WKY and SHR left ventricular relaxations (Figure 2).

The order of potency in augmenting the contractions, measured as the concentration that produced a 30% augmentation, was bretylium (3×10^{-7} M) > 4-aminopyridine (4×10^{-4} M) > tetraethylammonium (3×10^{-3} M). The blockers that augment force with minimal effects on relaxation probably have the best profile for the treatment of heart failure. Thus we determined % augmentation/% lengthening ratios. For tetraethylammonium, 4-aminopyridine and bretylium, these ratios were not significantly different on WKY and SHR left ventricles or between 50 and 90% relaxations values. The ratios were independent of the concentration of tetraethylammonium and bretylium and were $2{\cdot}03 \pm 0{\cdot}49$ (n = 8) and $3{\cdot}06 \pm 0{\cdot}52$ (n = 8), $P < 0{\cdot}05$, respectively. The ratios were greater with the higher (10^{-3} and 3×10^{-3} M) than the lower concentrations (10^{-4} and 3×10^{-4} M) of 4-aminopyridine and were $4{\cdot}23 \pm 0{\cdot}84$ and $2{\cdot}22 \pm 0{\cdot}57$ (n = 8), $P < 0{\cdot}05$, respectively.

None of the concentrations of tetraethylammonium, 4-aminopyridine or bretylium tested induced arrhythmias either alone or in the presence of isoprenaline.

Portal vein

Tetraethylammonium, 4-aminopyridine and bretylium were tested on eight portal veins from 14-week-old WKY rats, and had similar results on each. Tetraethylammonium at 10^{-6} M, 4-aminopyridine at 10^{-6} M or bretylium at 10^{-4} M did not alter the contractions of portal veins. Tetraethylammonium at 10^{-5} M increased the duration but did not alter the amplitude of the contractions, and at 10^{-3} M increased the duration and amplitude (Figure 5). 4-Aminopyridine at 10^{-5}–10^{-4} M had no effect on the amplitude but prolonged the contractions, and at 10^{-3} M also increased the amplitude (Figure 5). These effects of tetraethylammonium and 4-aminopyridine were not altered by pre-treatment with prazosin at 10^{-8} M for 60 min.

Small arteries

The WKY and SHRs were age-matched at 14 weeks, and were weight-matched (data not shown).

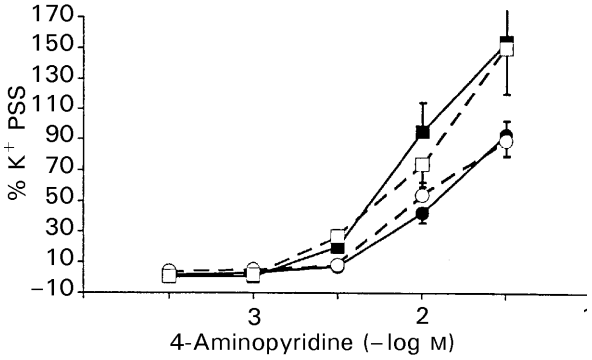

Figure 6. The effect of 4-aminopyridine on the intralobar pulmonary and mesenteric arteries of 14-week-old WKY and SHRs. Contractions from the intralobar pulmonary arteries of WKY (●) and SHRs (○), and the mesenteric arteries of WKY (■) and SHRs (□) are calculated as a percentage of the K^+PSS response, and plotted against the negative logarithm of the molar concentration of 4-aminopyridine. Each value is the mean ± s.e.m. from five or six preparations.

The SHRs had higher tail cuff pressures: WKY 131 ± 1 mmHg (n = 9), SHR 183 ± 2 mmHg (n = 7), $P < 0{\cdot}01$.

Tetraethylammonium at $\leq 3 \times 10^{-2}$ M had no effect on the intralobar pulmonary arteries. Tetraethylammonium at 10^{-2} and 3×10^{-2} M contracted the WKY by 19 ± 14 and 26 ± 17% (n = 4) and the SHR mesenteric arteries by 6 ± 3 and 21 ± 6% (n = 5), respectively, and these values were not significantly different between WKY and SHR. 4-Aminopyridine at 3×10^{-4}–3×10^{-2} M contracted the intralobar pulmonary and mesenteric arteries, and these contractions were not different between WKY and SHR (Figure 6). Bretylium at $\leq 3 \times 10^{-2}$ M had no effect on intralobar pulmonary or mesenteric arteries.

Discussion

As hypertrophy in humans is usually associated with chronic hypertension, the 12-month-old SHR is a realistic, but rarely used, model of human hypertrophy. We show that at 12 months the SHR has left ventricular hypertrophy, impaired contractility of the left ventricle and an increased in-vivo pulse rate. 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 begins to fail.

The association of the ability of potassium-channel blockers to increase the action potential duration with the prevention of re-excitation of the cardiac muscle is well known. The present study shows that there is also an association between the potassium-channel blocker-induced increase in action potential duration and a positive inotropic effect. Thus similar concentrations of tetraethylammonium, 4-aminopyridine and bretylium prolonged the action potential and had a positive inotropic effect on the left ventricle.

Potassium-channel blockers including bretylium are used as anti-arrhythmic agents (Roden 1996). The beneficial effects of potassium-channel blockers in arrhythmias are due to their ability to prolong the action potential and prevent re-excitation. However pro-arrhythmic effects of potassium-channel blockers have also been described, presumably due to prolongation of the action potential (Roden 1996). Thus a possible problem with the use of potassium-channel blockers as inotropic agents is that they may cause excessive prolongation of action potentials, which is associated with early after-depolarization and arrhythmias such as torsades de pointes (Singh et al 1993). One of the most interesting findings of the present study was that none of the drugs tested was arrhythmogenic, even in the presence of maximal concentrations of isoprenaline on the left ventricle. The highest concentration of 4-aminopyridine tested, 10^{-2} M, was toxic as it abolished action potentials and contractions. This toxic effect of 4-aminopyridine is probably due to excessive potassium-channel blockade preventing repolarization.

The positive inotropic effects of tetraethylammonium, 4-aminopyridine and bretylium on the left ventricle are probably due to their potassium-channel blocking activities on cardiac muscle as they occur in the presence of guanethidine to block the release of noradrenaline from noradrenergic nerves. Some potassium-channel blockers (e.g. 4-aminopyridine) have been shown to release noradrenaline from nerves (Huang 1995). Thus it is possible that 4-aminopyridine is causing a positive inotropic effect by releasing noradrenaline from the left ventricle by a guanethidine-insensitive mechanism. To test this, we investigated the effects of 4-aminopyridine at 10^{-4} and 10^{-3} M on 12-month-old WKY left ventricular strip contractility in the presence of propranolol at 10^{-7} M. Propranolol will block any positive inotropic effects of noradrenaline released by 4-aminopyridine at β-adrenoceptors. In the presence of propranolol, 4-aminopyridine at 10^{-4} and 10^{-3} M augmented the peak force of contraction by 28 and 82% (n = 5), respectively (Doggrell & Wilkie, unpublished observations), and these values are not significantly different from those in the absence of propranolol. Thus it seems likely that the positive inotropic effects of 4-aminopyridine, in the presence of

guanethidine, are due to potassium-channel blockade on the cardiac muscle.

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 relaxations of 12-month-old SHR left ventricles were prolonged, and the potassium-channel blockers further prolonged relaxation. Of the blockers tested, high concentrations of 4-aminopyridine and bretylium had the largest % augmentation/% lengthening ratios, which means they may have a more favourable profile than tetraethylammonium which has a lower ratio.

To be useful in the treatment of heart failure, the inotropic effect must be maintained in the presence of cardiac hypertrophy. The effects of tetraethylammonium and bretylium were similar, but the effects of 4-aminopyridine were reduced on the left ventricle of 12-month-old SHRs compared to WKY rats. This suggests that the potassium channels which 4-aminopyridine, but not those that tetraethylammonium or bretylium interacts with, are altered in hypertrophy. Previous studies have shown that there is diminished transient outward potassium current in cardiac hypertrophy (Boyden & Jeck 1995). However, the results from the few studies investigating the effects of hypertrophy on the other potassium channels are not clear-cut. Thus, there are reports of both increases and decreases in the inward rectifier current, and there may be a decrease in the density of the delayed rectifier in the hypertrophied myocyte (Boyden & Jeck 1995). As the responses to bretylium, a selective inhibitor of the delayed outward rectifier, are not altered, it seems likely that the function of the delayed outward rectifying potassium channel is not altered in hypertrophied left ventricle of 12-month-old SHRs. The responses to tetraethylammonium, which predominantly blocks the inward rectifying and delayed outward rectifying channels (Cook 1990), are also not altered. Thus it is probable that the function of the inward rectifying potassium channel is not altered in the hypertrophied left ventricle of 12-month-old SHRs. 4-Aminopyridine blocks the transient outward, the ultrarapid component of the delayed outward and the Na^+-activated potassium channel of cardiac tissue (Doggrell et al 1998). However the ability of 4-aminopyridine to prolong the action potential in cardiac tissue is predominantly due to blockade of the transient outward rectifying potassium channel (Berger et al 1995; Firek & Giles 1995). Thus it seems likely that the decreased inotropic response to 4-aminopyridine on the 12-month-old SHR left ventricle strip represents a loss in function of the transient outward rectifying potassium channel in hypertrophy.

Tetraethylammonium and 4-aminopyridine are known to contract blood vessels by blocking Ca^{2+}-activated- and -delayed outward rectifying potassium channels, respectively (Halliday et al 1995). In the present study tetraethylammonium and 4-aminopyridine contracted the portal vein and mesenteric arteries, and 4-aminopyridine also contracted the intralobar pulmonary artery. The effects on the portal vein were maintained in the presence of prazosin, indicating that the responses to tetraethylammonium and 4-aminopyridine were not due to the release of noradrenaline from noradrenergic nerves acting at β-adrenoceptors. Thus it seems likely that tetraethylammonium and 4-aminopyridine are contracting the portal vein by blocking the blocking Ca^{2+}-activated and -delayed outward rectifying potassium channels, respectively.

It seems likely that there are differences in the characteristics of the cardiac and vascular delayed outward rectifying potassium channels. Thus bretylium blocks the cardiac channel to exert a positive inotropic effect but does not seem to block the vascular delayed outward rectifying channel as it has no effect on the blood vessels. As vasoconstriction is a detrimental effect in heart failure, potassium-channel blockers that have this effect, such as tetraethylammonium and 4-aminopyridine, are not likely to be to be useful in heart failure. In contrast potassium-channel blockers that are cardiac selective, such as bretylium, would have positive inotropic effects without detrimental effects on blood vessels.

The non-specific blockers, tetraethylammonium and 4-aminopyridine, clearly do not have any clinical potential because of their widespread effects including vasoconstriction. The main aim of this study was not to test individual agents, but to test the rationale that potassium-channel blockade is a potential positive inotropic mechanism for the treatment of heart failure. The results of this study support this, in that potassium-channel blockade was associated with positive inotropic effects that were maintained in hypertrophy without arrhythmogenic effects. Recently many newer, more selective, potassium-channel blockers have been developed for the treatment of cardiac arrhythmias, many of which have reasonable oral bioavailability (Doggrell et al 1998). Most of these newer agents have not been tested for positive inotropism and potential in the treatment of heart failure (Doggrell et al 1998). As the results of the present study indicate that the blocking of certain potassium channels may be associated with positive inotropism without detrimental effects on blood vessels,

further studies of the potential of potassium-channel blockers for the treatment of heart failure are indicated.

Acknowledgements
This project was supported by the Auckland Medical Research Foundation.

References

Berger, F., Borchard, U., Hafner, D., Kammer, T., Weis, T. (1995) Modulation of action potential duration by inhibition of the transient outward current in sheep cardiac Purkinje fibers. Basic Res. Cardiol. 90: 185–191

Boyden, P. A., Jeck, C. D. (1995) Ion channel function in disease. Cardiovasc. Res. 29: 312–318

Cook, N. S. (1990) In: Potassium Channels: Structure, Classification, Functional and Therapeutic Potential. Harwood Halsted Press, Great Britain

Colatsky, T. (1995) Antiarrhythmic drugs: where are we going? Pharmaceut. News 2: 17–23

Doggrell, S. A., Brown, L. (1998) Rat models of hypertension, cardiac hypertrophy and failure. Cardiovasc. Res. 39: 89–105

Doggrell, S. A., Nand, V., Brown, L. (1998) Positive inotropic responses to potassium channel blockers. Curr. Res. in Ion Channel Modulators 3: 77–86

Dorigo, P., Santostasi, G., Fraccarollo, D., Maragno, I. (1994) Inotropic agents in development. Exp. Opin. Invest. Drugs 3: 457–468

Firek, L., Giles, W. R. (1995) Outward currents underlying repolarization in human atrial myocytes. Cardiovasc. Res. 30: 31–38

Halliday, F. C., Aaranson, P. L., Evans, A. M., Gurney, A. M. (1995) The pharmacological properties of K^+ currents from rabbit isolated aortic smooth muscle cells. Br. J. Pharmacol. 116: 3139–3198

Huang, Y. (1995) 4-Aminopyridine-induced phasic contractions in rat caudal epididymis are mediated through release of noradrenaline. Eur. J. Pharmacol. 280: 231–234

Katz, A. M. (1991) In: Katz, A. M. (ed.) Physiology of the Heart. Raven Press, New York

Mulvany, M. J., Halpern, W. (1976) Mechanical properties of vascular smooth muscle cells in situ. Nature 260: 617–619

Nand, V., Doggrell, S. A., Barnett, C. W. (1997) The effects of veratridine on the action potentials and contractility of right and left ventricles from normo- and hypertensive rats. Clin. Exp. Pharmacol. Physiol. 24: 570–576

Roden, D. M. (1996) Antiarrhythmic drugs. In: Hardman, J. G., Limbird, L. E., Molinoff, P. B., Ruddon, R. W., Gilman, A. G. (eds) Goodman & Gilman's The Pharmacological Basis of Therapeutics. 9th edn, McGraw-Hill, Auckland, pp 839–874

Singh, B. N., Ahmed, R. (1994) Class III antiarrhythmic drugs. Curr. Opin. Cardiol. 9: 12–22

Singh, B. N., Ahmed, R., Sen, L. (1993) Prolonging cardiac repolarization as an evolving antiarrhythmic principle. In: Escande, D., Standen, N. (eds) Potassium Channels in Cardiovascular Medicine. Springer-Verlag, France, pp 247–272