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Effects of Tedisamil on Cardiovascular Tissues Isolated from Normo- and Hypertensive Rats

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Background: This study was undertaken to characterize the effects of tedisamil on isolated rat cardiovascular tissues, and identify actions that could be beneficial or detrimental in the treatment of cardiac disease.

Results: Tedisamil prolonged the Wistar Kyoto normotensive rat (WKY) left ventricular action potential and augmented the force of contraction of left ventricle strips. On the 12-month-old SHR model of cardiac hypertrophy, the augmenting effects of tedisamil at 10^{-6} and 3×10^{-6} M were reduced. On the 21-month-old SHR model of heart failure, the augmenting effects of tedisamil at 10^{-6} and 3×10^{-6} M were further reduced. The augmenting effect of tedisamil at 10^{-5} M was reduced to 47%. The rate of the right atrium of 16- to 17-month-old WKY was reduced by tedisamil at 10^{-5} and 10^{-4} M, and tedisamil had a similar effect on the SHR right atrium. Tedisamil at 10^{-6}—3×10^{-5} M contracted the portal veins of WKY and aortae of 12-month-old WKY and SHR.

Conclusions: The positive inotropic and negative chronotropic effects of tedisamil in the rat, which are partially or fully maintained in hypertrophied or failing myocardium would be beneficial in the treatment of heart failure. In contrast, the vasoconstrictor action of tedisamil will be detrimental in heart failure.

Key words: tedisamil, positive inotropism, SHR hypertrophy/failure, negative chronotropism, vasoconstriction.

Reducing heart rate is associated with an improvement in myocardial function during exercise, and in the presence of coronary artery stenosis. Thus bradycardic drugs are antiischemic and have potential in the treatment of ischemic heart disease and heart failure (1).

Tedisamil is a bradycardic agent that is in clinical trial for angina (2,3) and heart failure (4). Inhibition of the transient outward potassium current (I_{to}) of human atrial myocytes (5) probably underlies the bradycardic effect of tedisamil.

Tedisamil also inhibits the delayed rectifying potassium current (1_K) of rat and human ventricles (rat, 6; human, 5), and prolongs action potentials (rat, 7; human, 5). Tedisamil is antiarrhythmic in rat models of arrhythmias (7,8). I_K inhibitors are commonly used clinically in the treatment of cardiac arrhythmias (9,10). Prolonging the action potential is often associated with a positive inotropic effect. Preliminary reports of positive inotropism with tedisamil on isolated animal heart preparations have been made (11,12). There are no reports as to whether the positive inotropism with tedisamil is maintained in disease. Bradycardia, antiarrhythmic effects, and positive inotropism would be beneficial in the treatment of heart failure.

The aim of this study was to characterize the effects of tedisamil on isolated rat cardiovascular tissues, and identify actions that could be beneficial or detrimental in the treatment of cardiac disease. First, we investigated the effects of tedisamil on the action potentials of left ventricles isolated from Wistar Kyoto normotensive rats (WKY), and showed a prolongation of the potentials. Second, we determined whether tedisamil was a positive inotrope. Isolated cardiac muscles are used to define positive inotropic responses since

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loading conditions can be well controlled, in contrast to studies in the whole animal. Thus we have studied the effects of tedisamil on the force responses of WKY left ventricles, and show positive inotropism. These studies were performed in the absence and presence of β-adrenoceptor stimulation to determine whether tedisamil was pro-arrhythmic. Third, we determined whether the positive inotropic effects 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 21-month-old spontaneously hypertensive rats (SHRs). Because hypertrophy in humans is usually associated with chronic hypertension, the SHR at 12 months is a realistic model of human hypertrophy (13). Twenty-one-month-old SHRs are in cardiac failure (14). A preliminary report of the left ventricular data has been presented to the Australasian Section of the International Society for Heart Research (15). Fourth, we ascertained whether the bradycardic effects of tedisamil were retained in the presence of hypertrophy by studying the effects on the heart rate of the right atrium isolated from 16 to 17-month-old WKY and SHRs.

To our knowledge, there have been few studies of the effects of tedisamil on isolated blood vessels. In a recent clinical study of tedisamil as a bradycardic agent in the treatment of heart failure, tedisamil was unexpectedly shown to increase blood pressure (4). In the final part of our study, we determined whether tedisamil produced vasoconstriction by studying the effects of tedisamil on the isolated rat portal vein, aorta, intralobar pulmonary, and mesenteric arteries.

Methods

Rats

Breeding pairs of WKY 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 two to a cage with free access to standard rat chow and water. The University of Auckland Animal Ethics Committee approved animal protocols (Helsinki guidelines).

Beginning of the Experiments

Rats were stunned and exsanguinated. The aorta, portal vein, lungs, mesenteric bed, and/or heart was rapidly removed and placed in Krebs solution that was saturated with 5% CO_2 in oxygen and at 37°C. The free wall of the left ventricle or the right atrium was immediately 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; $CaC1_2$, 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 and atria experiments, the Krebs solution 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. At the end of experiments the atria, and the free wall of the left and right ventricle and septum were separated, blotted, and weighed. In all experiments mean values ± SEM were determined. Tests of significance between two groups were made by student 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 unpaired *t* test.

Recording of the Action Potentials from the Electrically Driven Rat Ventricle

The previous method was followed (16,17). This method has the same equilibration times with tedisamil as our contractility protocol. 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 with Krebs solution that had been vigorously bubbled with 5% CO_2 in oxygen before entering the recording chamber. Tissues were equilibrated for 60 minutes before the tip of a 3M KCl-filled microelectrode was inserted into a cell close to the surface of the tissue. The tissues were then stimulated at 2 Hz, 5-msec duration and 30V, and the resulting action potentials were amplified via a microelectrode preamplifier and displayed on an oscilloscope. After 3 minutes of stimulation, three of the recorded action potentials from a single cell were stored for analysis before the stimulator was turned off. Tissues either remained untreated or had tedisamil added to the Krebs solution perfusing the tissue. After a 15-minute equilibration, during which about 125-mL of drug-free or tedisamil containing solution superfused the tissue, a further period of 6-minute stimulation and recording of three action potentials was undertaken from the same cell. This procedure was repeated at 15-minute intervals for 60 minutes. Then the tissue either remained untreated or was treat-

ed with a higher concentration of tedisamil, and the procedure repeated. The diastolic potential, peak amplitude, and action potential duration at 50% and 90% repolarization were measured.

Measurement of Blood Pressure and Heart Rate

Twelve- and 21-month-old WKY and/or SHRs were weighed and then the tail cuff (systolic) blood pressure was measured using a tail plethysmograph (IITC Life Sciences Model 29). To do this the rats were placed in a Perspex holding cylinder and left in the dark for 30 minutes, 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 mm Hg 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 previous method (16,17) was followed with left ventricles from 12- and 21-month-old WKY and SHRs. Five strips were prepared from the 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 guanethidine and atropine-containing Krebs solution being vigorously bubbled with 5% CO_2 in oxygen and allowed to equilibrate for 75 minutes. Stimulation at 2 Hz (5-msec duration, 30V) was commenced and after 3 to 6 minutes, 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-minute cycle until an isoprenaline maximal response was obtained. Stimulation was then stopped and three ventricle strips were treated with differing concentrations of tedisamil while the other strip remained untreated. Strips were superfused with approximately 500 mL of Krebs solution over 75 minutes before a second challenge to cardiac stimulation and isoprenaline. This procedure was repeated with the tedisamil-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 msec-onds. The force responses of the second and third challenges from untreated tissues were less than the first challenge, and our data was calculated to take this into consideration. The force responses just prior to the second and third challenges to isoprenaline were calculated as a percentage of the force responses to stimulation prior to the first challenge with isoprenaline. 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 a percentage of the maximal response to the first challenge to stimulation and isoprenaline, and compared. When the maximal combined responses were not significantly different between tedisamil-treated and untreated tissues the combined data were normalized. If the maximal combined responses were significantly different, the combined data was calculated as a percentage of the maximum response during the first challenge; that is, before treatment, to illustrate the change in maximum response with tedisamil.

Recording of Rate from Right Atrium

The right atria from 16- to 17-month-old WKY 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 minutes during which about 250 mL of Krebs solution superfused the tissue. Rate was recorded with a Grass tachygraph and recorded on a Grass polygraph. The cumulative addition of tedisamil (10^{-7}, 10^{-6} M etc.) occurred on an 8-minute cycle.

Recording of Contractions from the Aorta

Endothelium-intact aortic rings from 12-month-old WKY and SHRs were prepared and suspended under 1.5 g tension in 5-mL organ baths and equilibrated for 60 minutes, 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 8 channel Grass polygraph (model 79B). Tissues were contracted by the addition of 90 mM KCl, and then washed by superfusing the tissue with 500 mL Krebs solution over 60 minutes. When testing the effects of drugs on the KCl-contracted tissue, the aortic rings were contracted by the addition of KCl at 15 mM until a plateau response was reached. Quiescent or KCl-contracted

tissues were cumulatively challenged with tedisamil on a 5-minute cycle or, when a response was observed, until the response was maximal.

Recording of Contractions from Portal Vein

The previous method (16,17) was followed. Portal veins (about 12 mm) derived proximally to the liver of 14-week-old WKY were mounted longitudinally under 1 g tension in 5-mL organ baths containing Krebs solution and allowed to equilibrate for 30 minutes. 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 to 30 minutes. Then a cumulative exposure to tedisamil on a 5-minute cycle was made. When studying the effect of prazosin on the responses to tedisamil, prazosin was added 60 minutes prior to the challenge to tedisamil. The amplitudes of the final three contractions before the addition of each concentration of tedisamil were measured, and the contractions were averaged. Responses were calculated as a percent change of the spontaneous contractile activity, and were corrected for changes in untreated tissues.

Recording of Contractions from Intralobar Pulmonary and Mesenteric Arteries

The previous method (16,17) was followed. The contractions of the intralobar pulmonary and mesenteric arteries of 14-week-old WKY were recorded in the Mulvany myograph containing Krebs solution at 37°C. After equilibration for 1h the pulmonary arteries was set up under the equivalent of 15 mm Hg and the mesenteric arteries from the WKY and SHRs under 60 and 80 mm Hg, respectively. The tissues were equilibrated for 15 minutes before two challenges with K+PSS (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 minutes before a cumulative challenge to tedisamil on a 3-minute cycle or until a maximum response was obtained. Contractions were calculated as a percentage of K+PSS response.

Drugs

The drugs used in this study were tedisamil (donated by Kalie-Chemie Pharmaceuticals), prazosin (donated by Pzifer Ltd), atropine sulphate, guanethidine sulphate, and (-)-isoprenaline bitartrate (Sigma Chemical Co).

Results

Effects of Tedisamil on Left Ventricular Action Potentials

The action potentials from 14-week-old WKY left ventricles had a mean diastolic membrane potential of –81 mV, amplitude of 96 mV, and APD_{50} and $_{90}$ values of 19 and 56 msec, respectively. These values did not change in 3 to 4 hours of testing (data not shown). Tedisamil at 10^{-7} M had no effect on the action potentials (Table 1). Tedisamil at 10^{-6} and 10^{-5} M had no effect on diastolic membrane potential or amplitude of left ventricular action potentials (data not shown). For 15 minutes, tedisamil at 10^{-7} and 10^{-6} M had no effect on the duration of the action potential (see Table 1). After 30 minutes, prolongation was observed with tedisamil at 10^{-6} and 10^{-5} M, and this prolongation was further increased after 45 minutes (see Table 1). A similar prolongation was observed with tedisamil at 10^{-7} or 10^{-6} M after 45 and 60 minutes (see Table 1), and this indicates that the effects of these concentrations of tedisamil are at equilibrium.

Tedisamil at 10^{-6} and 10^{-5} M for 60 minutes caused a greater prolongation at the APD_{50} (action potential duration to 50% repolarization) than at the APD_{90} level (see Table 1).

SHR Model of Hypertrophy and Heart Failure

The WKY and SHRs were age-matched at 12 and 21 months, and were weight-matched at 12 months (Table 2). Twenty-one-month-old SHRs were lighter than age-matched WKY (see Table 2). Twelve- and 21-month-old SHRs had higher tail cuff blood pressures than age-matched WKY (see Table 2). Heart rates were higher by 31 and 205 beats/min in 12- and 21-month-old SHRs than age-matched WKY (see Table 2). Heart weights were calculated as percent body weight to negate differences due to changes in body weight. 12-month-old SHR had greater left ventricles and septum, but not right ventricles or atria, weights than WKY (see Table 2). Twenty-one-month-old SHRs also had greater right ventricle and atria weights than age-matched WKY (see Table 2).

Effects of Tedisamil on Left Ventricular Contractility

Tedisamil at 3×10^{-8} to 3×10^{-7} M had no effect on left ventricular contractility (data not shown). Tedisamil at 10^{-6} to 10^{-5} M also had no effect on the T_P of the contractions of left ventricles of 12- and 21-month-old WKY or SHRs, (data not shown). Tedisamil at 10^{-6} to

Table 1. Effects of Tedisamil on 14-week-old WKY Left Ventricle Action Potential Duration

Group	APD_{50} (msec) % Increase		APD_{90} (msec) % Increase	
Untreated	18.7 ± 0.5		55.6 ± 1.5	
Tedisamil at 10^{-7} M				
60 min	18.8 ± 0.4		55.4 ± 1.3	
Tedisamil at 10^{-6} M				
15 min	19.0 ± 0.5		56.8 ± 1.6	
30 min	25.6 ± 1.0*		64.6 ± 0.6*	
45 min	30.2 ± 1.0*		76.1 ± 1.5*	
60 min	30.2 ± 1.4	60 ± 6	76.2 ± 1.4	32 ± 6†
Tedisamil at 10^{-5} M				
15 min	30.8 ± 0.9		76.7 ± 0.2	
30 min	35.9 ± 1.7*		85.3 ± 0.2*	
45 min	41.3 ± 1.7*		92.2 ± 0.1*	
60 min	41.5 ± 1.4	122 ± 5	92.2 ± 1.0	60 ± 5†

Each value is the mean ± SEM. Number of rats in each group = 4.
*$p < 0.05$ with previous time point.
†$p < 0.05$ with APD_{50} increase.

Table 2. Characteristics of 12- and 21-Month-Old WKY and SHRs

Characteristic	12-WKY	12-SHR	21-WKY	21-SHR
Age (days)	369 ± 7 (8)	374 ± 8 (8)	638 ± 12 (6)	650 ± 14 (6)
Weight (g)	453 ± 12 (8)	466 ± 15 (8)	458 ± 15 (6)	361 ± 10 (6)*
Heart rate (beats/min)	325 ± 13 (8)	355 ± 12 (8)*	280 ± 8 (6)	401 ± 7 (6)*
Tail cuff BP (mmHg)	129 ± 5 (8)	200 ± 10 (8)*	130 ± 7 (6)	198 ± 8 (6)*
Heart weights as percent body weight $\times 10^{-2}$				
Heart	24.09 ± 0.34 (8)	29.01 ± 0.21 (8)*	25.31 ± 0.92 (6)	49.23 ± 0.73 (6)*
Left ventricle	10.47 ± 0.15 (8)	13.41 ± 0.74 (8)*	11.19 ± 0.18 (6)	21.57 ± 0.28 (6)*
Septum	6.06 ± 0.12 (8)	8.41 ± 0.23 (8)*	6.21 ± 0.88 (6)	11.96 ± 0.94 (6)*
Right ventricle	5.69 ± 0.12 (8)	5.73 ± 0.22 (8)	5.96 ± 0.21 (6)	11.39 ± 0.28 (6)*
Atria	1.87 ± 0.08 (8)	1.46 ± 0.21 (8)	1.95 ± 0.10 (6)	4.31 ± 0.09 (6)*

Each value is the mean ± SEM (number of rats).
*$p < 0.05$, with age-matched WKY.

10^{-5} M significantly augmented the peak force of WKY left ventricle contractions to electrical stimulation ($p < 0.05$) and this produced an augmentation of the submaximal ($p < 0.05$), but not maximal, responses in the presence of isoprenaline; Fig. 1. The augmentation effect of tedisamil force to electrical stimulation was similar on the 12- and 21-month-old WKY (data not shown). On the 12-month-old SHR left ventricle, the augmenting effect of tedisamil at 10^{-6} and 3×10^{-6}, but not 10^{-5} M, was reduced (Fig. 2). On the 21-month-old SHR left ventricle, the augmenting effects of tedisamil at 10^{-6} and 3×10^{-6} M were further reduced, and the augmenting effect of tedisamil at 10^{-5} M was also reduced (see Fig. 2).

On WKY left ventricles, tedisamil at 10^{-6} to 10^{-5} M prolonged relaxation (see Fig. 2), and the effect on the TR_{50} was significantly greater extent than on the TR_{90} values; $p < 0.05$ at each concentration. The prolonging effect with tedisamil was similar on 12- and 21-month-old WKY (data not shown). The effect of tedisamil at 10^{-6} and 3×10^{-6}, but not 10^{-5} M, was reduced on the 12-month-old SHR left ventricle (see Fig. 2). Tedisamil at 10^{-6} and 3×10^{-6} M exerted positive inotropic effects on the 21-month-old SHR left ventricle without prolonging the TR_{90} values (see Fig. 2). The ability of tedisamil at 10^{-5} M to prolong relaxation was reduced on the 21-month-old SHR left ventricle (see Fig. 2).

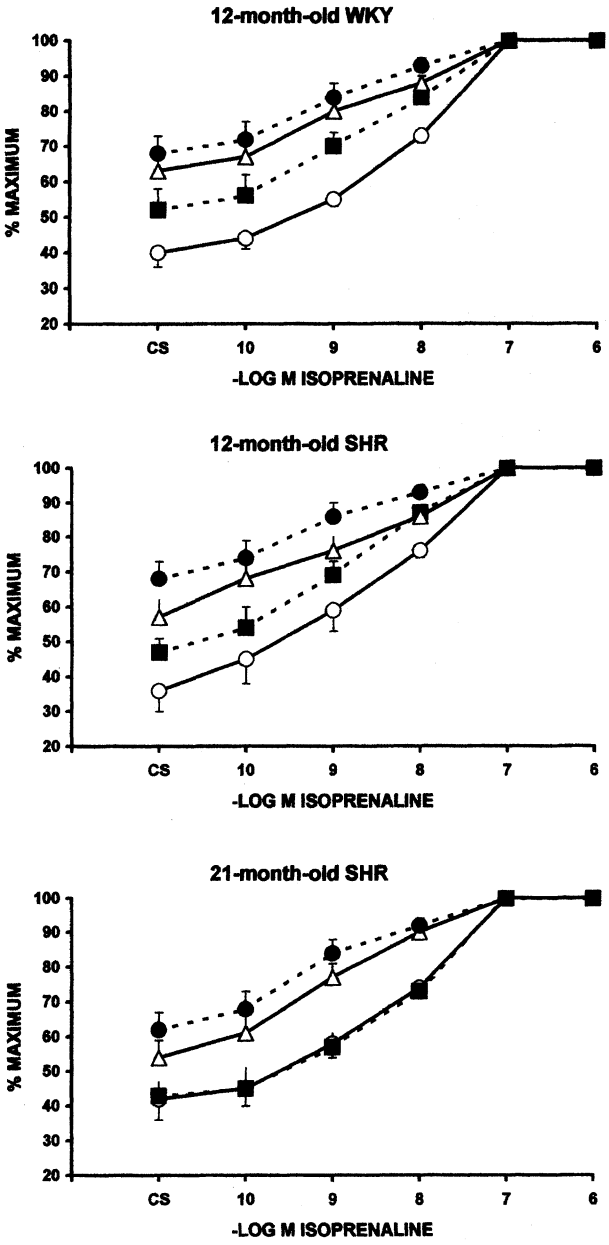

Fig. 1. The effects of tedisamil on the contractions of left ventricles from 12-month-old Wistar Kyoto (WKY) rats (top), 12-month-old spontaneously hypertensive rats (SHRs, middle) and 21-month-old SHRs (bottom). Contractions from untreated ventricles (○) and from ventricles treated with tedisamil at 10^{-6} (■), 3×10^{-6} (△) and 10^{-5} M (●). Contractions were calculated as a percentage of the maximal 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 ± SEM from six to eight preparations.

The augmenting effects of tedisamil at 10^{-5} M on the cardiac stimulation response was not significantly different to that at 3×10^{-6} M on the WKY left ventricle (see Fig. 2), and this suggests that the response is near maximal. In contrast the augmenting effect of tedisamil at 10^{-5} M was greater than that of 3×10^{-6} M on the SHR left ventricle (see Fig. 2). Thus it was possible that the maximum augmenting effect had not been achieved with tedisamil at 10^{-5} M on the SHR left ventricle.

In the final series of experiments, we tested the effects of tedisamil at 3×10^{-5} and 10^{-4} M on the SHR left ventricular contractility, and showed that these higher concentrations caused attenuation. The four SHRs used were about 23 months old (686 days ± 3) and had right ventricle hypertrophy (right ventricle/body weight ratio of 8.82 ± 0.66, $p < 0.05$ with 21-month-old WKY). Tedisamil at 3×10^{-5} and 10^{-4} M had no effect on the T_P of the contractions (data not shown). Tedisamil at 3×10^{-5} and 10^{-4} M attenuated the peak force of the 23-month-old SHR left ventricle contractions to electrical stimulation ($p < 0.05$), and this produced an attenuation in the presence of isoprenaline, $p < 0.01$ (Fig. 3). The TR_{50} and TR_{90} values of 23-month-old SHR left ventricle contractions were 59.4 ± 3.3 msec and 105.0 ± 8.0 msec (4), respectively. Tedisamil at 3×10^{-5} M had no effect on these TR values (data not shown). Tedisamil at 10^{-4} M shortened the TR_{50} and TR_{90} values of 23-month-old SHR left ventricle contractions to 46.7 msec ± 1.8 and 84.4 msec ± 7.4 (4), respectively ($p < 0.05$ with untreated).

Effect of Tedisamil on Right Atrium Rate

Tedisamil was tested on the isolated right atrium from four WKY (488 days ± 1) and four SHRs (503 ± 1) with similar resting rates; WKY, 248 beats^{-1} ± 4 (4), SHR, 234 ± 12 (4). The SHR right atrium had hypertrophy [right atrium/body weight ratio, SHR; 1.630 ± 0.072 (4), WKY; 0.937 ± 0.032 (4), $p < 0.01$]. Tedisamil at 10^{-7} and 10^{-6} M had no effect, and at 10^{-5} and 10^{-4} M decreased the rate of the WKY right atrium (Fig. 4). Tedisamil had similar effects on the rate of the SHR right atrium (see Fig. 4).

Effects of Tedisamil on Blood Vessels

The portal vein of 14-week-old WKY has spontaneous contractile activity, and the amplitude of these contractions was increased by tedisamil at 10^{-8}–10^{-4} M (Fig. 5). The tedisamil-induced contractions were not altered by prazosin at 10^{-8} M (n = 4, see Fig. 5).

The quiescent aortae of 12-month-old WKY and SHR were contracted by tedisamil at 10^{-6} to 3×10^{-5} M, and these contractions were reversed by tedisamil

at 10^{-4} M (see Fig. 5). The responses to tedisamil developed slowly, and it was about 16 minutes before a maximum response was reached to each concentration. The KCl-contracted WKY aortae were further contracted by tedisamil at 3×10^{-7} to 10^{-5} M (n = 4).

Tedisamil at 10^{-8} M to 3×10^{-3} M has no effect on the quiescent mesenteric and intralobar arteries of 14-week-old WKY and SHRs (n = 6).

Discussion

Tedisamil prolongs the action potential of rat ventricular myocytes (intact; 7, isolated; 6), dogs ventricles (18) and human atrial and ventricular fibers (5). In our study we quantified the effects of tedisamil on the WKY left ventricle action potential duration, and showed that tedisamil prolonged the APD_{50} to a greater extent than the APD_{90}. This confirms the findings of Beatch and associates (7) that showed that tedisamil had a greater effect on the APD_{50} than the APD_{75} in rat ventricles.

On the WKY left ventricle, we have shown that drugs that predominantly inhibit $I_K/I_{to\text{-}s}$ prolong the APD_{90} to a greater extent than the APD_{50} (clofilium, 19; d-sotalol, 20; dofetilide, 21). Tedisamil blocks $I_{to}/I_{to\text{-}f}$ and $I_K/I_{to\text{-}s}$, and prolongs the APD_{50} to a greater extent than the APD_{90}. This suggests that inhibition of $I_{to}/I_{to\text{-}f}$ is associated with the ability to prolong the APD_{50} to a greater extent than the APD_{90}. Excessive prolongation of the WKY left ventricle APD_{90} with clofilium (335% at 10^{-5} M, 19) and d-sotalol (230% at 10^{-5} M, 20) may predict proarrhythmic activity. In contrast, modest prolongation of the APD_{90} with dofetilide (50% at 10^{-7} M, 21) and tedisamil (60% at 10^{-5} M, this study) probably suggest a low incidence of proarrhythmia.

In the rat, I_{to} is the predominant ventricular repolarization current (22) whereas in the human the predominant ventricular repolarization current is I_K. Thus the rat is probably not the best model to test the electrophysiological effects of tedisamil. The action potential prolonging effect of tedisamil (0.3 mg/kg IV) in human ventricles has been shown to be quite modest, 16% at the APD_{90} (23), which suggests that tedisamil is unlikely to be proarrhythmic.

We have studied the effects of tedisamil on the action potentials of WKY ventricles. Action potentials are prolonged in hypertrophy and heart failure (SHR 24,25: human 26). It is possible that tedisamil has proarrhythmic actions on diseased, but not normal, ventricular action potentials. Thus, further studies of the effects of tedisamil on hypertrophied and failing ventricles may be indicated.

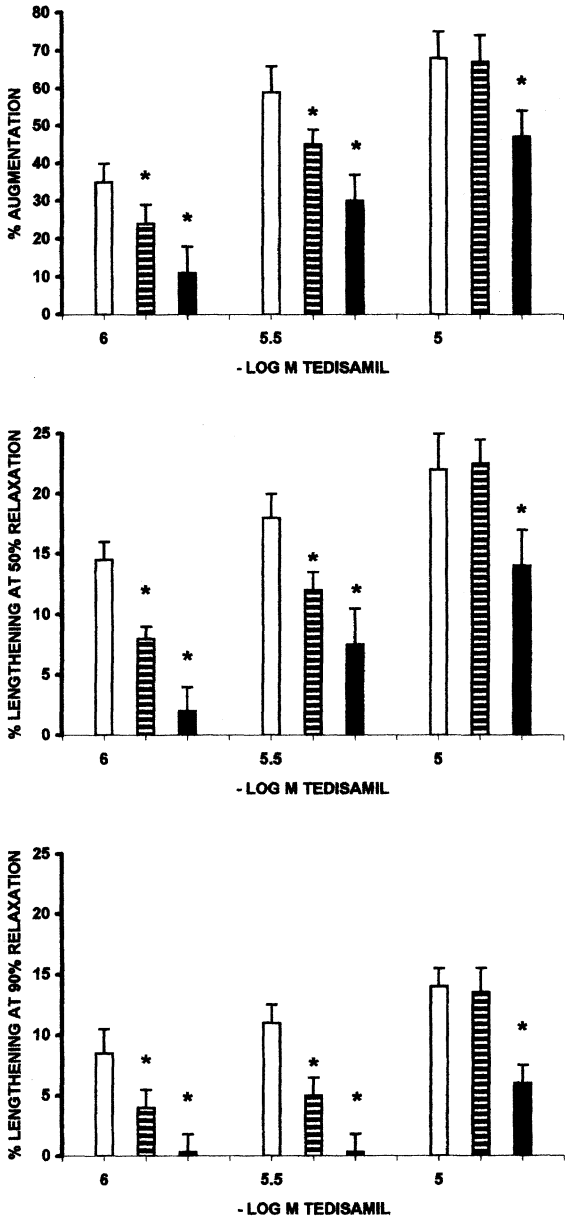

Fig. 2. The effects of tedisamil on the contractions of left ventricles from 12-month-old Wistar Kyoto (WKY) rats (□), 12-month-old spontaneously hypertensive rats (SHRs, hatched columns) and 21-month-old SHRs (■). Top: percentage augmentation of force; middle and bottom: percentage lengthening of TR_{50} and TR_{90}, respectively, are plotted against the negative logarithm of the molar concentration of tedisamil. Each value is the mean ± SEM from six to eight preparations. *$p < 0.05$, unpaired t-test, with the same concentration of tedisamil on 12-month-old WKY rats.

Potassium channel blockers are used as antiarrhythmics (27). The beneficial effects of potassium channel blockers in arrhythmias may be due to their ability to prolong the action potential and prevent re-excitation.

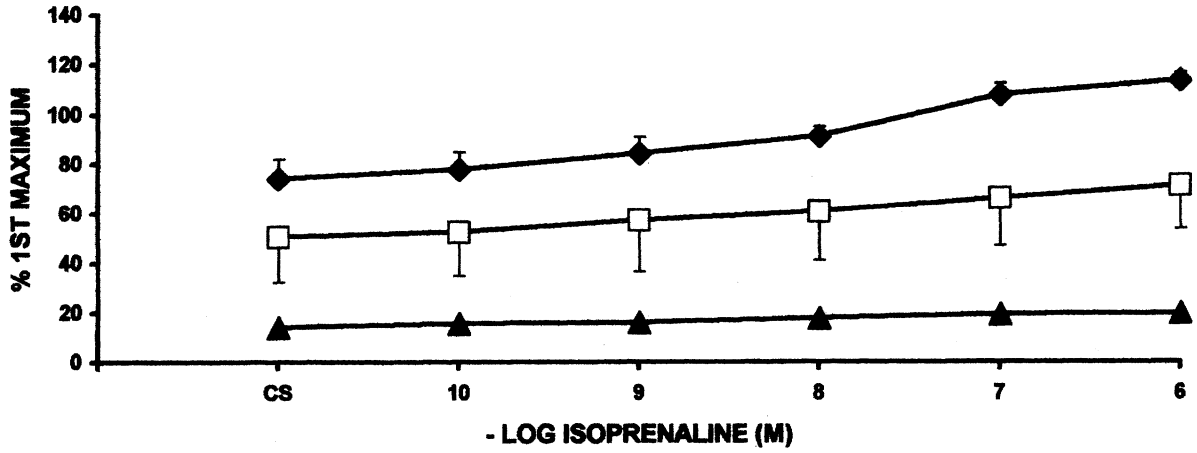

Fig. 3. The effects of tedisamil on the contractions of left ventricles from 23-month-old spontaneously hypertensive rats. Contractions from untreated ventricles (◆) and from ventricles treated with tedisamil at 3×10^{-5} (□) and 10^{-4} M (▲). Contractions were calculated as a percentage of the maximal 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 ± SEM from four preparations.

However pro-arrhythmic effects of potassium channel blockers have also been described, presumable due to excessive prolongation of the action potential or the appearance of after-depolarization. This is a potential problem with the use of potassium channel blockers as positive inotropes. In our contractility studies, tedisamil alone or in the presence of isoprenaline did not induce arrhythmias in left ventricles from normotensive or hypertensive rats. Antiarrhythmic but not pro-arrhythmic effects have been reported with tedisamil in animal studies (rat; 7,8, rabbit; 28, dog; 29,30). The antiarrhythmic activity of tedisamil in myocardial ischemia is probably related to blocking K_{ATP} channels (28,29) in addition to I_K and I_{to}. In healthy volunteers, tedisamil induced reverse rate-dependent QT interval prolongation but this was not excessive (31).

Our study was not designed to determine which ion channels tedisamil was interacting with. To date, only two outward rectifying potassium currents have been characterized in rat ventricles, I_{to} or $I_{to\text{-}f}$ and I_K or $I_{to\text{-}s}$, and tedisamil has a tenfold selectivity for I_K (EC_{50}, 5×10^{-7} M) over I_{to} (6). Tedisamil also inhibits I_{to} in human atria and ventricles (5). Indirect evidence suggests that tedisamil may also inhibit K_{ATP} as tedisamil has been shown to reverse the effects of pinacidil, a K_{ATP} channel opener, in the coronary artery occluded model of ventricular fibrillation in the rabbit heart (28). High concentrations of tedisamil, greater than 2×10^{-5} M, also inhibit I_{Na} (32). In the presence of oxygenation, the K_{ATP} channels of the rat ventricle are firmly closed as no effects are observed with cromakalim, a K_{ATP} channel opener (33). The present study was performed with vigorous bubbling of the rat left ventricle strips with $95\%O_2/5\%CO_2$ which will promote the closure of the K_{ATP} channels, and make it unlikely that tedisamil is blocking K_{ATP} channels. It is more likely that the effects of tedisamil at 10^{-6} to 10^{-5} M in the present study are due to inhibiting I_K and I_{to}. We have previously demonstrated that 4-aminopyridine, which inhibits I_{to} and I_K, prolongs the action potential and has a positive inotropic effect on the isolated left ventricles of rat ventricles (16). The attenuating effects of tedisamil at 3×10^{-5} and 10^{-4} M on the SHR left ventricle may be due to inhibition of I_{Na}.

Preliminary studies have indicated that tedisamil is a positive inotrope in the rat (11). The present study is the first to show that tedisamil has a marked inotropic effect on the ventricle strip from the rat.

One of the main aims of this study was to determine whether the positive inotropic effects of tedisamil were maintained in the presence of advanced cardiac hypertrophy and failure by studying the effects of tedisamil on the SHR left ventricles. As hypertrophy and then failure in humans is usually associated with chronic hypertension, the 12- and 21-month-old SHRs are realistic models of human hypertrophy and failure, respectively (13,14). We show that 12- and 21-month-old SHRs have left ventricular hypertrophy.

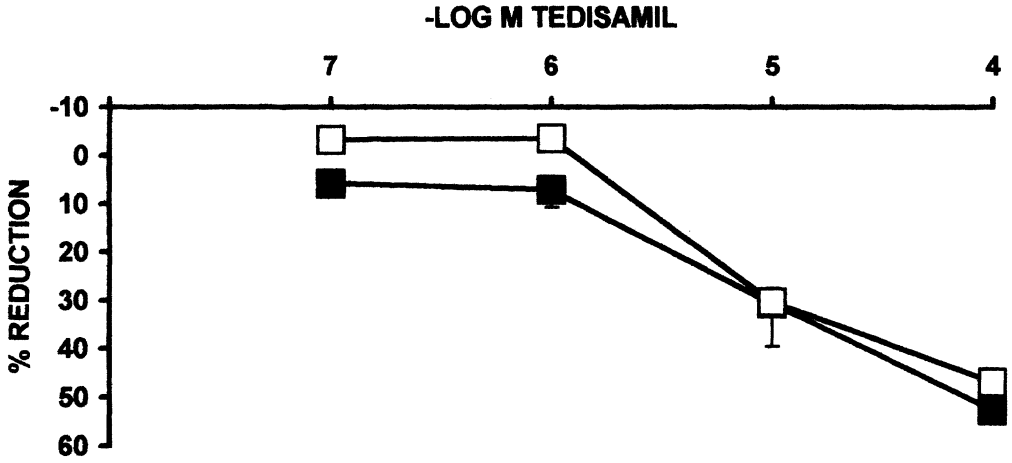

Fig. 4. The effects of tedisamil on the rate of beats of the right atrium of 16- to 17-month-old Wistar Kyoto rats (□) and spontaneously hypertensive rats (■). Rate was calculated as percentage difference from untreated and plotted against the negative logarithm of the molar concentration of tedisamil. Each value is the mean ± SEM from four preparations.

The SHR as a model of heart failure has been characterized by Bing and associates (14). Of many markers tested, the most consistent marker of the SHR in failure was right ventricular hypertrophy (14). All of our SHRs at 21 months had right ventricular hypertrophy. Bing and colleagues (14) have divided their 18- to 24-month-old SHRs into two groups SHR-F (failing), which have right ventricular hypertrophy; and SHR-NF (nonfailing), which do not have right ventricular hypertrophy. Our 21-month-old SHRs are a homogenous SHR-F group as they all had right ventricular hypertrophy. We have recently demonstrated that the heart failure in our SHRs is associated with left ventricle wall thickening and dilation, decreased magnitude of left ventricle contractions to electrical stimulation and isoprenaline, and a prolongation of contraction and relaxation of the left ventricle (17).

In the present study the inotropic effects of tedisamil were reduced in hypertrophy. We have previously shown that the inotropic responses to $CaCl_2$ are not altered on the 12-month-old SHR (25), and this shows that there is no nonspecific loss of left ventricular contractility at this time. I_{to} but not I_K is reduced in SHR hypertrophy (34,35). Thus it seems likely that the reduced effect of tedisamil in SHR hypertrophy may be due to reduced I_{to}.

There is further reduction in the inotropic effect of tedisamil in SHR heart failure. This reduction will be partly due to the reduction in I_{to}. In cardiomyocytes from 18-month-old SHR heart failure, the I_{to} remained reduced with no effect on I_K (34). We have recently shown that the effects of selective I_K blockers such as azimilide (17), dofetilide (21) and d-sotalol (20) are reduced on 20-22-, but not 12-month-old SHR left ventricles. From these data, we postulated that there might be further alterations in potassium currents between 18 and 20 months in the SHR with an alteration in the function of I_K in failing SHR left ventricles at 20 months. If this is the case, the further reduced inotropic effect of tedisamil in SHR heart failure may be due to changes in function of I_K. Further ion channel studies with greater than 18-month-old SHRs are needed to elucidate whether I_K is altered as heart failure progresses.

An alternative possibility that may account for some or all of the further loss of effectiveness of tedisamil in heart failure, is that there is a nonspecific loss in contractility in SHR heart failure. We have previously shown that the maximum responses to $CaCl_2$ on the 18-month-old SHR left ventricle are reduced (25), and this suggests that there is a nonspecific loss of contractility in SHR heart failure.

Tedisamil has a marked inotropic effect on WKY left ventricles. Although a considerable amount of this activity is lost in hypertrophy and heart failure, the positive inotropic effect remaining in heart failure is substantial, 47% with tedisamil at 10^{-5} M. To date, the inotropic effects of tedisamil on isolated normal and

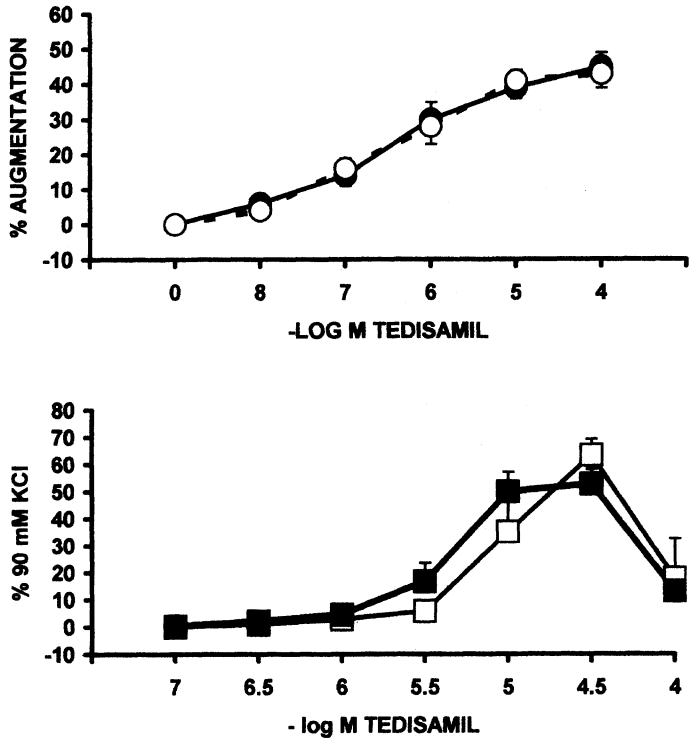

Fig. 5. The effects of tedisamil on portal vein of 14-week-old Wistar Kyoto (WKY) rats (top) in the absence (●) and presence of prazosin at 10^{-8} M (○), and, bottom, on the aortae of 12-month-old WKY (□) and spontaneously hypertensive rats (■). Portal vein contractions were calculated as percentage difference from untreated, and aorta contractions as a percentage of the response to 90 mM KCl. Responses were plotted against the negative logarithm of the molar concentration of tedisamil. Each value is the mean ± SEM from four or eight preparations.

diseased human heart have not been determined. As tedisamil is such an effective positive inotrope in a rat model of heart failure, studies of its possible inotropic effects on human heart should be undertaken.

Drugs that augment force with minimal prolonging effects on relaxation probably have the best profile for the treatment of heart failure. In our study of SHR heart failure, tedisamil at 10^{-6} and 3×10^{-6} M was a positive inotrope without prolonging TR_{90}. We have previously tested azimilide (17), dofetilide (21), and d-sotalol (20) in the SHR model of heart failure, and shown that these drugs are positive inotropes and prolong relaxations. Thus it seems that tedisamil has a more favorable profile on SHR left ventricular relaxation than some of the other blockers of potassium channels.

By inhibiting I_{to}, that are present in the sinoatrial node and ventricles (9), tedisamil not only has a positive inotropic effect but also has a negative chronotropic effect. The bradycardic effect of tedisamil has been demonstrated in animal models of disease (11,29,30), in healthy volunteers (31), and in human cardiac disease (coronary artery disease, 2, 3; congestive heart failure due to dilated cardiomyopathy, 4). The present study shows that the bradycardic effect of tedisamil is maintained in the presence of hypertrophy of the SHR right atrium.

Tedisamil at greater than or equal to 10^{-6} M inhibits I_K and Ca^{2+}-activated potassium channels ($I_{K(Ca)}$) of the guinea-pig portal vein (36,37). We have previously demonstrated that inhibition of I_K with 4-aminopyridine or $I_{K(Ca)}$ with tetraethylammonium is associated with contraction of the rat portal vein and aorta (16). In the present study, the ability of tedisamil to contract the rat portal vein and aorta may be due to inhibition of I_K and/or $I_{K(Ca)}$. The portal veins used in the present study were from 14-week-old WKY, and further studies are required to determine whether the portal vein contracting effects of tedisamil are maintained on the portal vein in senescence.

Clinical studies with tedisamil have not shown positive inotropy with tedisamil. Tedisamil did not alter cardiac contractility in patients with coronary disease (2). Tedisamil contracts the rat aorta (this study) and increases human total peripheral resistance (4). Increases in afterload are associated with a decreased cardiac output in heart failure. It is possible that in heart failure, tedisamil is having two opposing effects on cardiac output. First, a positive inotropic effect on the heart, which would increase cardiac output. Second, tedisamil is having a vasoconstrictor effect to increase afterload and decrease cardiac output. The vasoconstrictor effects of tedisamil could be overcome by the concomitant use of vasodilators. Further studies of the effects of tedisamil in combination with vasoditators on cardiac output may be warranted.

To our knowledge, there have been few studies measuring plasma concentrations of tedisamil in humans. In healthy volunteers receiving 100 mg tedisamil twice daily, the peak plasma concentration of tedisamil was about 2×10^{-6} M (25). The positive inotropic and vasoconstrictor effects of tedisamil in our study, occur at 10^{-6} M and are likely to be clinically relevant. Although concentrations of tedisamil greater than 10^{-6} M were required to exert a bradycardic effect in our rat right atrium study, the bradycardic effect of tedisamil has been shown to occur clinically (2–4,31).

In conclusion, the positive inotropic and negative chronotropic effects of tedisamil in the rat, which are partially or fully maintained in the presence of heart failure, would be beneficial in the treatment of heart failure. In contrast, the vasoconstrictor action of tedisamil may be detrimental in heart failure. Further testing of tedisamil in combination with vasodilators in animal models of heart failure, and in human heart failure are suggested.

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