JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 1097–1102

Received February 21, 2002
Accepted April 25, 2002
ISSN 0022-3573

Effects of BDF 9198 on left ventricular contractility in advanced spontaneously hypertensive rats with heart failure

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Abstract

In the first part of this study, we characterized 24-month-old Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs), their heart weights, and the responses of the isolated left ventricles to electrical stimulation. In the main part of the study, we tested whether the positive inotropic effects of BDF 9198, which prevents the closure of the cardiac sodium channel, were present in senescence and heart failure. Thus, we studied the effects of BDF 9198 on the left ventricle strips of 24-month-old WKY rats (senescence) and SHRs using contractility methods. In comparison with WKY rats, the left ventricles of 24-month-old SHRs were hypertrophied and had prolonged times to peak contraction. BDF 9198 (10^{-8} to 10^{-6} M) was a positive inotrope on the left ventricles of WKY rats, with a maximum augmenting effect of 122% with BDF 9198 at 10^{-7} M. The magnitude of the augmenting effects of BDF 9198 were reduced in SHR heart failure, with a maximum augmenting effect of 26% at 10^{-7} M. BDF 9198 at 10^{-6} M attenuated the responses of the SHR left ventricle to electrical stimulation. In conclusion, the potential of drugs that prevent closure of the sodium channel as positive inotropes in the treatment of heart failure should be further considered.

Introduction

A variety of positive inotropes (β-adrenoceptor agonists and calcium sensitizers) are used in the short-term hospital treatment of decompensated heart failure, but most of these are not orally active. Of the positive inotropes, only digoxin is presently used for the long-term treatment of heart failure, as a variety of other agents (β-adrenoceptor agonists, phosphodiesterase inhibitors) have been shown to increase mortality. Digoxin does not affect mortality, but it does decrease hospitalizations for heart failure (Rich et al 2001). Thus, improved approaches to positive inotropic agents for the treatment of heart failure should be considered. Prolonging the cardiac action potential is often associated with a positive inotropic effect, and this prolongation can be achieved by opening or inhibiting the closure of sodium channels (Doggrell & Brown 1997).

The cardiac sodium channel is the H1 channel, and the opening of this tetrodotoxin-resistant channel has been shown to produce the rapid depolarization (phase 0) of the cardiac action potential (Balser 2001). The opening of this sodium channel in turn leads to the opening of voltage-dependent calcium channels and calcium entry into the cell. Increasing intracellular Ca^{2+} concentrations lead to an increase in the force of contraction.

Cardioselective inhibitors of the H1 sodium channel inactivation, BDF 9148 and BDF 9198, prolong the opening of the sodium channel and are positive inotropes (BDF 9148 : Ravens et al 1991 ; Nand & Doggrell 1999 ; BDF 9198 : Muller-Ehmsen et al 1998 ; Yuill et al 2000). Poor bioavailability made BDF 9148 unsuitable for clinical use and stopped its development for clinical use, whereas BDF 9198 has improved bioavailability (Doggrell & Brown 1997).

There have been few studies to determine whether there are any changes in the structure or function of cardiac sodium channels in ageing and/or cardiac disease.

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Funding: Supported by a Project grant from the Auckland Medical Research Foundation.

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Although preliminary electrophysiological studies have indicated no change (Ten Eick et al 1992), functional studies indicate that there may be changes in hypertrophy and heart failure. On the hypertrophied left ventricle of the 6-month-old spontaneously hypertensive rat (SHR), the positive inotropic effects of BDF 9148 were reduced by 15–35%, but no further reduction was observed when the SHR went into the early stages of heart failure at 18 months (Nand & Doggrell 1999). It is not known whether the positive inotropic effects of BDF 9198 are maintained or reduced as SHR heart failure advances.

The first part of our study was a characterization of 24-month-old Wistar Kyoto (WKY) rats and SHRs, their heart component weights, and the responses of the isolated left ventricle to electrical stimulation. The main aim of our study was to determine whether BDF 9198 was a positive inotrope in the presence of advanced SHR heart failure. Thus, we describe the effects of BDF 9198 on the left ventricle of 24-month-old SHRs.

Materials and Methods

Rats

Breeding pairs of WKY rats and Okamoto SHRs were purchased from the Animal Resources Centre, Perth, WA, Australia, and then colonies of these rats were established in the Animal Resource Unit, Faculty of Medicine and Health Sciences, The University of Auckland. Adult rats were housed three to a cage, with free access to standard rat chow and water. The Animal Ethical Committee of the University of Auckland approved all procedures, which conform to the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health.

Measurement of blood pressure and heart rate

WKY rats and/or SHRs (24 months old) were weighed and then the tail cuff (systolic) blood pressure was measured using a tail plethysmograph (model 29; IITC Life Sci.). In order 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 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 (these were usually very similar) and were averaged.

Heart preparation

WKY rats or SHRs (24 months old) were stunned and exsanguinated. The heart was rapidly removed and placed in Krebs solution that was saturated with 5% CO_2 in oxygen, at 37°C, and the free wall of the left ventricle was excised. All experiments were performed in the presence of a modified Krebs solution (composition in mM: NaCl 116; KCl 5.4; $CaCl_2$ 2.5; $MgCl_2$ 1.2; NaH_2PO_4 1.2; $NaHCO_3$ 22.0; D-glucose 11.2) containing 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.

Recording contractions from the electrically driven rat ventricle

We have previously described the method for recording ventricular contractility (Nand & Doggrell 2000). Five strips were prepared from the left ventricle free wall. Four of the strips were mounted longitudinally between two platinum electrodes under 1 g tension in 5-mL organ baths in Krebs solution vigorously bubbled with 5% CO_2 in oxygen and allowed to equilibrate for 75 min. Ventricular contractility was recorded and displayed on a Grass polygraph/polyview system. After stimulation at 2 Hz for 6 min, isoprenaline at 10^{-10} M was added. The cumulative addition of isoprenaline (e.g. 10^{-9}, 10^{-8} M) 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 BDF 9198, while the other strip remained untreated or was treated with vehicle. 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 BDF 9198 tissues that were treated receiving higher concentrations of the drug, and the untreated tissue remaining untreated or being treated with vehicle.

The time to peak contraction (T_p), and times from maximal force to 50% (TR50) and 90% (TR90) relaxation were measured in ms. Maximal forces of contractions to cardiac stimulation and the maximal combined responses to cardiac stimulation and isoprenaline were measured as mg tension. The maximum responses to isoprenaline as mg tension were calculated by subtracting the cardiac stimulation response from the maximum combined response. In addition, the force response before the first challenge with isoprenaline was considered the 100% control, and the responses prior to the second and third challenges to isoprenaline were calculated as a percentage of this. If these force responses to cardiac stimulation between treated and untreated tissues were significantly different, the percentage difference between the values from the individual treated tissues and from the mean of the untreated tissues was also calculated.

The maximal combined responses to cardiac stimulation and isoprenaline from the first challenge to isoprenaline were also designated as the 100% control. The maximal responses during the second and third challenges to isoprenaline were calculated as a percentage of this, and compared. When the maximal

combined responses were not significantly different between BDF-treated and untreated tissues, indicating that BDF 9198 had no effect on isoprenaline maximums, the standard normalization procedure of expressing data as a percentage of maximum response of individual concentration–response curves was undertaken. When the maximal combined responses were significantly different between BDF-treated and untreated tissues, indicating that BDF 9198 had an effect on isoprenaline maximums, the data was calculated as a percentage of the maximum response of the first (untreated) concentration–response curves to illustrate the effect on maximal responses.

Heart characteristics

The free walls of the left and right ventricle, septum, and left and right atria were separated, blotted and weighed.

Statistical analysis

In all experiments, mean values ± s.e.m. were determined. Tests of significance between responses in the absence and presence of BDF 9198 were made by analysis of variance followed by Student's paired *t*-test in WKY and SHR ventricles. Comparisons between the inotropic effects of BDF 9198 on WKY and SHR ventricles were made by Student's unpaired *t*-test.

Drugs

The drugs used in this study were BDF 9198 (donated by Beiersdorf-Lilly GmbH) and atropine sulfate, guanethidine sulfate, and (−)-isoprenaline bitartrate (Sigma Chemical Co.). BDF 9198 at 10^{-2} M was dissolved in absolute ethanol.

Results

Rat and heart characteristics

The WKY rats and SHRs used were age-matched (Table 1). The SHRs had lesser bodyweights, but higher tail cuff pressures and pulse rates than the WKY rats (Table 1). To take into account that 24-month-old SHRs had lower bodyweights than age-matched WKY rats, heart weights were calculated as mg (g bodyweight)$^{-1}$. As mg (g bodyweight)$^{-1}$, 24-month-old SHRs had greater heart, left ventricle, septum, right ventricle and atria weights than age-matched WKY rats (Table 1).

Table 1 Rat and heart characteristics.

	WKY rats	SHRs
Age (days)	742±16	707±34
Bodyweight (g)	428±12	385±8*
Tail cuff pressure (mmHg)	141±5	213±6*
Pulse rate (beats min^{-1})	328±8	370±5*
Weights as mg (g bodyweight)$^{-1}$		
Heart	2.67±0.08	5.43±0.36*
Left ventricle	1.18±0.08	2.56±0.26*
Septum	0.71±0.05	1.13±0.08*
Right ventricle	0.61±0.05	0.94±0.13*
Atria	0.161±0.008	0.779±0.008*

WKY, Wistar Kyoto; SHRs, spontaneously hypertensive rats. Each value is the mean ± s.e.m. of six rats. *$P < 0.05$ compared with age-matched WKY rats.

Contractions

Stimulation at 2 Hz (5 ms, 30 V) caused contractions of the left ventricle strips that were augmented by isoprenaline. Comparison of the responses from 24-month-old WKY and SHRs ventricles showed that the T_p values were prolonged on the left ventricles of SHRs: WKY rat, 55.7 ms ± 3.5 (n = 6); SHR, 63.5 ms ± 4.8 (n = 6), $P < 0.05$. The magnitude of the peak contractions to cardiac stimulation was similar on the 24-month-old WKY and SHR left or right ventricles: WKY rat, 227 mg ± 32 (n = 6); SHR, 256 mg ± 29 (n = 6), not significantly different. On 24-month-old SHR left ventricles, the response to isoprenaline was almost abolished (isoprenaline maximum; WKY rat, 121 mg ± 12 (n = 6); SHR, 26 mg ± 13 (n = 6), $P < 0.05$). The TR values were similar on 24-month-old WKY and SHRs left ventricles: TR50; WKY rat, 53.7 ms ± 3.3 (n = 6); SHR, 59.3 ms ± 4.4 (n = 6): TR90; WKY rat, 104.1 ms ± 5.8 (n = 6); SHR 102.3 ± 5.5 (n = 6). The vehicle, 0.03% ethanol, did not alter the contractions. With repeated stimulations of the left ventricle at 90-min intervals over 3 h, the contractions to cardiac stimulation were reduced, the time course of responses was not altered, and the magnitude of the responses to isoprenaline increased.

Effects of BDF 9198 contractility

BDF 9198 at 3×10^{-9} M had no effect on WKY rat left ventricular contractility. BDF 9198 at 10^{-8} to 3×10^{-7} M had no effect on the T_p of WKY rat left ventricular contractions, but at 10^{-6} M it shortened the T_p from 55.7 ms ± 3.5 (n = 5) to 47.0 ms ± 2.4 (n = 5) ($P < 0.05$). The contraction force to cardiac electrical stimulation of the WKY rat left ventricle was augmented with BDF 9198 at 10^{-8} to 10^{-7} M, and this was associated with an augmentation of the submaximal, but not maximal, responses in the presence of isoprenaline (Figure 1). Higher concentrations of BDF 9198, 3×10^{-7} to 10^{-6} M, augmented the contraction force to cardiac stimulation, but reduced the maximal response to isoprenaline in the

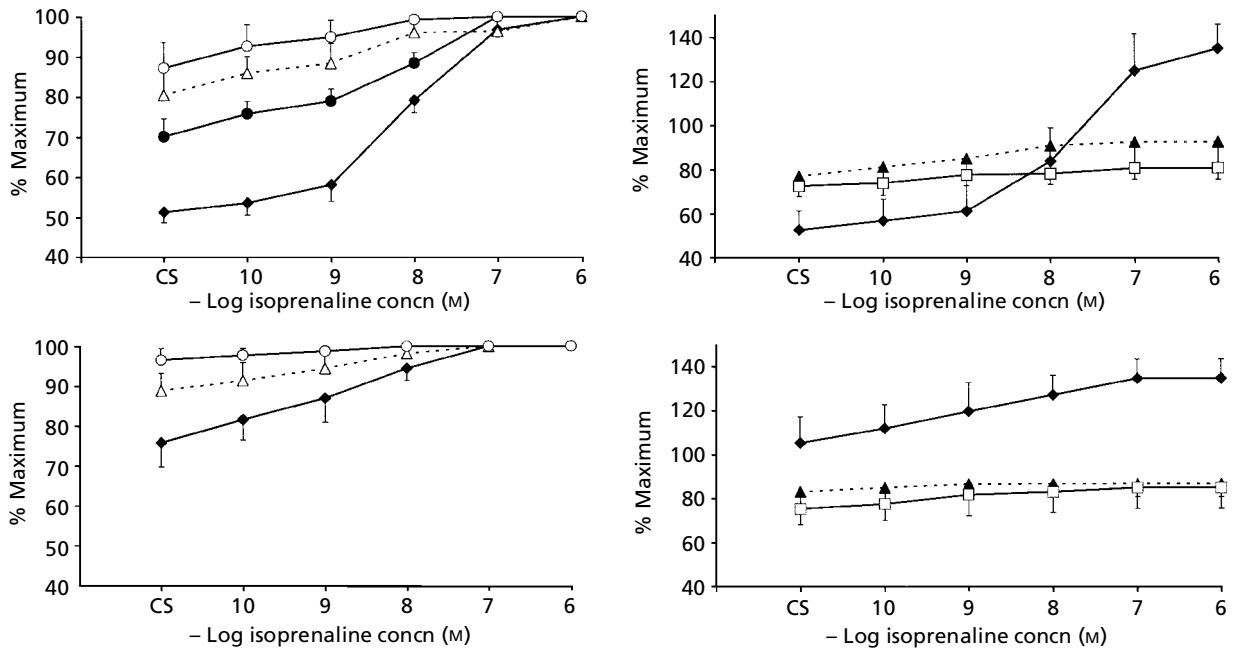

Figure 1 Effects of BDF 9198 on the response of the left ventricles of 24-month-old Wistar Kyoto rats (top) and spontaneously hypertensive rats (bottom). Responses from untreated tissues (◆) and tissues treated with BDF 9198 at 10^{-8} (●), 3×10^{-8} (△), 10^{-7} (○), 3×10^{-7} (▲), and 10^{-6} M (□). When BDF 9198 had no effect on the maximum response, responses were calculated as % maximum (left), and when BDF 9198 attenuated the maximum responses to cardiac stimulation and isoprenaline, responses were calculated as % maximum during the initial untreated challenge (right). Responses were 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 six preparations.

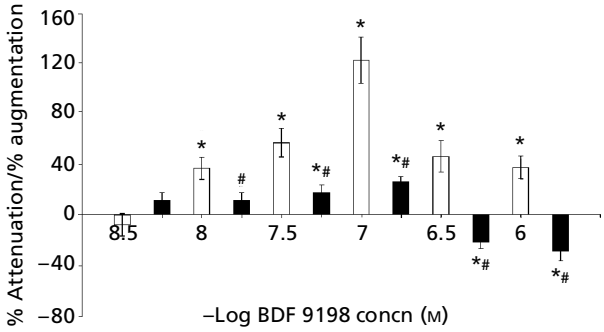

Figure 2 Percentage augmenting (positive values) and attenuation (negative values) effects of BDF 9198 on the left ventricles from Wistar Kyoto (WKY) rats (open columns) and spontaneously hypertensive rats (closed columns). Each value is the mean±s.e.m. from six preparations. *$P < 0.05$ compared with untreated preparation, #$P < 0.05$ compared with WKY rats.

presence of cardiac stimulation (Figure 1). BDF 9198 at 3×10^{-9} to 10^{-6} M had no effect on the relaxation times of the WKY rat left ventricle.

On SHR left ventricles, the time to peak was not altered by BDF 9198 at 3×10^{-9} to $\times10^{-6}$ M. The peak force to cardiac stimulation of the SHR left ventricle was not altered by BDF 9198 at 10^{-8} M, but was augmented by BDF 9198 at 3×10^{-8} to 10^{-7} M, producing an augmentation of the submaximal, but not maximal, responses in the presence of isoprenaline (Figure 1). The SHR force responses were attenuated by higher concentrations of BDF 9198, 3×10^{-7} and 10^{-6} M (Figure 1). The times to relaxation of SHR left ventricles were not altered by BDF 9198 at 3×10^{-9} to 10^{-6} M.

On the WKY rats, the maximal augmentation of the cardiac stimulation response was observed with BDF 9198 at 10^{-7} M, and lesser effects were observed with BDF 9198 at 3×10^{-7} to 10^{-6} M (Figure 2). Augmentation was observed with similar concentrations of BDF 9198 on the WKY and SHR ventricles. However, the maximum augmentation observed with BDF 9198 on the SHR ventricles was much less than that observed on WKY rat ventricles (Figure 2). Attenuation was observed with the highest concentration of BDF 9198 tested (10^{-6} M) on the SHR, but not the WKY rat ventricles (Figure 2).

Discussion

Bing et al (1995) initially characterized the SHR as a model of heart failure; of many markers tested, the most consistent marker of the SHR in failure was right ventricular hypertrophy. They divided their 18 to 24-month-old SHRs into two groups; SHR-F (failing), which have right ventricular hypertrophy, and SHR-NF (non-failing), which do not have right ventricular hypertrophy. Our 18 to 24-month-old SHRs are a homogenous SHR-F group as they all have right ventricular hypertrophy (18 months: Nand & Doggrell

1999; 22 months: Nand & Doggrell 2000; 24 months: present study).

On the left ventricle, we have recently demonstrated that the heart failure in our 22-month-old SHRs is associated with wall thickening and dilation, a lesser magnitude of left ventricle contractions to isoprenaline, and a prolongation of the contraction and relaxation of the left ventricle (Nand & Doggrell 2000). Comparing the 22- (Nand & Doggrell 2000) and the 24-month-old data (present study) shows that at 24 months, the magnitude of SHR left ventricle contractions to isoprenaline have been further reduced, and the prolongation of the contraction of the left ventricle persists. However at 24 months, the relaxations of the WKY and SHR left ventricles have a similar duration. Comparison between 22 (Nand & Doggrell 2000) and 24 months (present study) shows that the relaxation times have increased in the WKY rat left ventricle. As a consequence of this age-related change in the WKY rat left ventricle relaxation, there is no longer a difference between the relaxations of the left ventricles of WKY and SHRs at 24 months.

It seems probable that the SHR left ventricle losses in maximum contractions to isoprenaline are due to selective impairment of the β-adrenoceptor contractile pathway, as there is no generalized impairment of contractility. Hypertension-induced hypertrophy or heart failure reductions in cardiac β-adrenoceptor responsiveness are well documented (Feldman 1987; Kompa et al 1999; Owen et al 1999; Willette et al 1999).

The main finding of the present study is that the magnitude of the maximum augmenting effects of BDF 9198 in SHR heart failure is much reduced. It is not known whether the magnitude of the augmenting effect is lost in human heart failure. In the studies of BDF 9198 in human heart failure (Muller-Ehmsen et al 1997, 1998; Schwinger et al 1999), the concentration–response curves to BDF 9198 on control and diseased hearts have been normalized (maximum response 100%), and such data do not give a comparison of the absolute magnitude of response.

With higher concentrations of BDF 9198 (3×10^{-7} and 10^{-6} M), the augmenting effects are reduced on the WKY and become attenuation on the SHR ventricles. On guinea-pig cardiomyocytes, BDF 9198 at 10^{-6} M prolonged I_{Na} without affecting L-type calcium current, or delayed and inward-rectifying potassium channels (Yuill et al 2000). Thus, it seems unlikely, unless there are species or age-related changes in the ion channel, that BDF 9198 is blocking the L-type calcium channel to attenuate responses. The mechanism underlying this reduction in augmentation/attenuation is not known. The augmenting effects on inotropic responses of a similar drug that prevents the closure of sodium channels, BDF 9148, are also reduced in SHR hypertrophy (Nand & Doggrell 1999). This suggests that there may be alterations in the sodium channels with hypertrophy/heart failure, and this should be further investigated.

In summary, we have shown that the positive inotropic effects of low concentrations of BDF 9198 are reduced, but present, in advanced SHR heart failure. Our studies also show that high concentrations of BDF 9198 attenuate responses in advanced SHR heart failure, which would be detrimental in heart failure.

In conclusion, BDF 9198, or other drugs that prevent the closure of the cardiac sodium channel, may have some potential as positive inotropes for the treatment of heart failure. However, further studies of the effects of differing concentrations of drugs that prevent the closure of sodium channels in models of heart failure should be undertaken before these drugs are further considered for clinical use.

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