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Inotropic responses to phosphodiesterase inhibitors in cardiac hypertrophy in rats

Oleg Osadchii*, Gavin Norton, Angela Woodiwiss

Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, University of the Witwatersrand Medical School, 7 York Road, Parktown, Johannesburg, 2193, South Africa

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Abstract

In the present study we sought to determine whether reduced contractile responses to phosphodiesterase inhibitors occur in the face of chronic cardiac hypertrophy associated with β-adrenergic inotropic downregulation. As compared to age-matched Wistar–Kyoto control rats, spontaneously hypertensive rats at 6–8 months of age exhibited a striking decrease in left ventricular inotropic responses induced by isoproterenol, a β-adrenoceptor agonist, in isolated, isovolumically contracting heart preparations. Despite profound β-adrenoceptor-mediated inotropic downregulation, similar contractile responses to the phosphodiesterase III selective inhibitors, amrinone and milrinone, the phosphodiesterase IV selective inhibitor, rolipram, and non-selective phosphodiesterase inhibitor, pentoxifylline, were detected in normal and hypertrophic heart preparations. Moreover, the inotropic potency of the cAMP analogue, 8-Br-cAMP, was increased in spontaneously hypertensive rats. These findings suggest that in chronic cardiac hypertrophy, contractile responses to phosphodiesterase inhibitors may be preserved despite marked reductions in inotropic responses to β-adrenoceptor agonists.

Keywords: Adrenergic downregulation; Phosphodiesterase inhibitor; cAMP; Myocardial contractility

1. Introduction

Pharmacological agents that activate the β-adrenoceptor-cAMP signaling pathway, such as β-adrenoceptor agonists or phosphodiesterase (PDE) inhibitors, are useful in providing hemodynamic stability in acute cardiac decompensation (Stevenson et al., 1998). Nevertheless, it is well-recognized that if this approach is employed in patients with preceding chronic heart failure, the therapeutic benefits of these agents may be reduced for at least two reasons. First, many patients with chronic heart failure receive β-adrenoceptor blockers as standard medical therapy (Waagstein et al., 1993), which results in the need to use β-adrenoceptor agonists at elevated doses that enhance stroke volume whilst simultaneously increasing heart rate and blood pressure (Lowes et al., 2001). Heart rate and blood pressure changes predictably increase cardiac loading conditions. In this regard, the use of a PDE inhibitor appears to produce a greater benefit than that of a β-adrenoceptor agonist (Lowes et al., 2001). Second, reduced inotropic responses to β-adrenoceptor agonists may transpire following downregulation of the myocardial β-adrenoceptors in heart failure (Bristow et al., 1982; Feldman et al., 1987; Bohm et al., 1988b), an effect that appears to also reduce the inotropic potency of PDE inhibitors (Feldman et al., 1987; Bohm et al., 1988b; Von der Leyen et al., 1991).

Abnormal β-adrenergic regulation of myocardial contractility is not necessarily a direct consequence of chronic heart failure. It is also noted in cardiac hypertrophy with well-preserved contractile function (Kumano et al., 1983; Bohm et al., 1988a, 1994; Castellano et al., 1993; Habuchi et al., 1995). As cardiac hypertrophy predisposes to coronary artery disease (Kannel et al., 1970), predictably it is likely to precede acute cardiac decompensation in a significant number of patients, thus requiring inotropic support. Whether similar decreases in contractile responses

* Corresponding author. Tel.: +27 11 717 2363/2354; fax: +27 11 717 2153.
E-mail address: woodiwissaj@physiology.wits.ac.za (O. Osadchii).

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to PDE inhibitors occur in cardiac hypertrophy without heart failure has not been established. Therefore, the aim of the present study was to determine the efficacy of contractile responses to PDE inhibitors in an animal model of cardiac hypertrophy well recognized as being associated with downregulation of the β-adrenergic signaling pathway.

2. Materials and methods

This study complies with the European Community Guidelines for the Care and Use of Experimental Animals, and was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (clearance number: 2003/77/3). Male normotensive Wistar–Kyoto control (WKY) rats and spontaneously hypertensive rats (SHR) at 6–8 months of age were used in these experiments.

2.1. Echocardiography and blood pressure measurements

In order to compare left ventricular function as measured in vivo between study groups, echocardiography was performed using a 7.5 MHz transducer and a Hewlett Packard Sonos 2000 sector scanner, as previously outlined (Chung et al., 1998; Woodiwiss et al., 2001; Norton et al., 2002). All measurements were obtained at 15 min after the induction of ketamine and xylazine anesthesia (75 and 15 mg/kg i.p., respectively). Left ventricular internal dimensions were measured according to the American Society for Echocardiography's leading edge method (Sahn et al., 1978). Measurements were made from three consecutive beats, and endocardial (FS_{end}) and midwall (FS_{mid}) fractional shortening determined as previously described (Chung et al., 1998; Norton et al., 2002). Left ventricular endocardial and midwall fractional shortening were utilized as indexes of chamber and myocardial function, respectively (Norton et al., 2002).

Invasive blood pressure was measured from carotid artery catheter assessments obtained at 15 min after the induction of ketamine and xylazine anesthesia.

2.2. Isolated, perfused heart preparations

Inotropic responses to pharmacological agents were assessed using techniques previously described (Norton et al., 1999). Rats were anesthetized with an intraperitoneal injection of ketamine and xylazine at the doses indicated above. Thereafter, the chest was opened, and the heart was quickly excised and placed in ice-cold physiological saline solution. The hearts were retrogradely perfused via the aorta at a constant flow with carefully filtered and warmed (37 °C) physiological saline solution saturated with 95%O_2 and 5%CO_2. The solution contained (in mM) 118.0 NaCl; 4.7 KCl; 2.5 $CaCl_2$; 25 $NaHCO_3$; 1.2 KH_2PO_4; 1.2 $MgSO_4$; and 10.0 glucose, and had a pH of 7.4. The coronary flow rate was measured by collecting venous effluent, and adjusted to be 10 ml/min per g of heart weight. To exclude rate-dependent variations in contractility, the cardiac preparations were paced at a constant frequency of 330 beats/min, with the voltage 10% above threshold, via platinum wire electrodes attached to the right atrium and the apex of the left ventricle. In order to record ventricular developed pressure, the left atrium was trimmed and a water-filled balloon-tipped cannula coupled to a Statham P23 pressure transducer, was introduced through the atrioventricular orifice into the left ventricular cavity. Left ventricular developed pressure was calculated as the difference between end-systolic and end-diastolic pressure, and was recorded on a Hellige polygraph. The maximum rates of left ventricular pressure development ($+dP/dt$) and relaxation ($-dP/dt$) were obtained using a differentiator (model 13-4616-71, Gould Instrument Systems, Valley View, OH) with a high-frequency cutoff set at 300 Hz.

We assessed contractile effects elicited by isoproterenol, a β-adrenoceptor agonist; amrinone and milrinone, two selective PDE III inhibitors; rolipram, a selective PDE IV inhibitor; pentoxifylline, a non-selective PDE inhibitor (Meskini et al., 1994); and 8-bromoadenosine 3′5′-cyclic monophosphate (8-Br-cAMP), which is a cell-permeable, PDE-resistant cAMP analogue. The agents used were dissolved either in saline (isoproterenol, pentoxifylline, 8-Br-cAMP) or dimethylsulfoxide (amrinone, milrinone, rolipram) and infused just proximal to the aortic cannula by means of a Harvard infusion pump (model 22M T/W). The rate of infusion was 0.3 ml/min, and the total duration of infusion of each concentration of each agent was 60 s. Concentration–response relations were constructed by assessing contractile responses to incremental concentrations of each pharmacological agent. Intervals of at least 5 min were allowed between infusions for left ventricular developed pressures to return to baseline values after a preceding pharmacological exposure. Prior to the infusions of active agents, we ascertained that the vehicle produced no contractile responses. Concentration–contractile response curves were constructed for all substances used in order to compare the efficacy (maximal inotropic response) and potency (EC_{50}) of inotropic agents in WKY rats and SHR.

To compare left ventricular function in WKY rats and SHR at matched coronary flow and heart rates, baseline left ventricular systolic pressure–volume relations were constructed in a random sample of rat hearts just prior to assessing contractile responses to pharmacological agents. In order to construct left ventricular systolic pressure–volume relations, the ventricular balloon volume was gradually increased by increments of 0.01 ml using a micromanipulator and left ventricular developed pressures were determined at each volume. Load-independent systolic chamber function was assessed for each heart by evaluating the linearised slope of the left ventricular developed pressure–volume relations (Woodiwiss et al., 2001; Norton et al., 2002). Load-independent measures of intrinsic

systolic myocardial function were assessed by constructing left ventricular developed stress–strain relations and comparing the slopes of the relationships (Norton et al., 2002). Left ventricular developed stress and strain values were calculated using previously described equations (Weber et al., 1988).

2.3. Pharmacological agents

(±)-Isoproterenol hydrochloride, amrinone, milrinone, rolipram, pentoxifylline and 8-bromoadenosine 3′5′-cyclic monophosphate were purchased from Sigma (St. Louis, USA).

2.4. Analysis of data

Results are expressed as mean±S.E.M. The magnitude of the inotropic responses elicited by pharmacological interventions was expressed as a percent increase in left ventricular developed pressure. The concentrations of substances that produced 50% of the maximal contractile response (EC_{50}) were determined from regression analysis using logistic sigmoid function curves (log concentration vs. effect) (Norton et al., 1999), and are presented throughout as pEC_{50} values ($pEC_{50} = -\log_{10}EC_{50}$). The slopes of left ventricular developed pressure–volume and stress–strain relations in WKY rats and SHR were determined by linear regression analysis (Woodiwiss et al., 2001). The magnitude of inotropic responses elicited over the range of concentrations of each substance evaluated was assessed by repeated measures ANOVA (analysis of variance), followed by a Tukey–Kramer post hoc test. Comparisons of all variables between WKY rats and SHR were performed by an unpaired Student's *t*-test. Probability values <0.05 were considered to be significant.

3. Results

3.1. Cardiac weight, systolic blood pressure and chamber performance

Despite the lower body weights noted in SHR, they had higher absolute heart weights and left ventricular weights, as well as increased heart weight-to-body weight and left ventricular weight-to-body weight ratios as compared to WKY control rats (Table 1). Left ventricular weight-to-body weight ratio was increased by 20% in SHR as compared to the mean value for WKY rats.

Systolic blood pressure values were significantly higher in SHR in comparison to age-matched WKY rats (Table 1).

When assessing left ventricular systolic function and contractile responses to inotropic agents, coronary flow was adjusted to comparable values in heart preparations taken from WKY rats and SHR (10.2±0.3 and 9.7±0.2 ml/min per g of heart weight, respectively, $P=0.2$). Neither FS_{end}, FS_{mid} nor the slopes of the systolic pressure–volume and stress–strain relations were altered in SHR as compared to WKY control rats (Table 1 and Fig. 1). In addition, echocardiographic assessment showed similar values of left ventricular end-diastolic diameter in the two groups of rats (Table 1). Thus, there was no evidence of systolic dysfunction or left ventricular dilatation in SHR.

Table 1
Cardiac weight, systolic left ventricular function and blood pressure in spontaneously hypertensive and Wistar–Kyoto control rats

	WKY rats	SHR
Body weight (g)	382±7 ($n=37$)	358±4[a] ($n=28$)
Heart weight (g)	1.51±0.03 ($n=37$)	1.62±0.02[a] ($n=28$)
Left ventricular weight (g)	1.12±0.02 ($n=37$)	1.27±0.02[b] ($n=28$)
Heart weight-to-body weight ratio (g/kg)	3.97±0.03 ($n=37$)	4.54±0.04[b] ($n=28$)
Left ventricular weight-to-body weight ratio (g/kg)	2.94±0.03 ($n=37$)	3.54±0.03[b] ($n=28$)
Systolic blood pressure (mm Hg)	144±6 ($n=10$)	194±4[c] ($n=20$)
Left ventricular FS_{end} (%)	62±2 ($n=10$)	55±3 ($n=17$)
Left ventricular FS_{mid} (%)	34±2 ($n=10$)	29±2 ($n=17$)
Left ventricular EDD (cm)	0.66±0.03 ($n=10$)	0.66±0.02 ($n=17$)
Slope of the left ventricular systolic pressure–volume relation (mm Hg/ml)	1448±213 ($n=21$)	1713±198 ($n=28$)
Slope of the left ventricular systolic stress–strain relation (g/cm^2)	1192±106 ($n=21$)	1231±92 ($n=28$)

WKY—Wistar–Kyoto; SHR—spontaneously hypertensive rats; FS_{end}—endocardial fractional shortening; FS_{mid}—midwall fractional shortening; EDD—end-diastolic diameter.
[a] $P<0.01$ versus Wistar–Kyoto rats.
[b] $P<0.001$ versus Wistar–Kyoto rats.
[c] $P<0.0001$ versus Wistar–Kyoto rats.

3.2. Cardiac contractile response

Both for WKY rats and SHR, similar baseline left ventricular developed pressures and $+dP/dt$ values were noted when comparing groups in whom hearts were exposed to different agents (Table 2).

A striking reduction in the magnitude of the inotropic response to the β-adrenoceptor agonist, isoproterenol, occurred in SHR as compared to WKY control rats (Fig. 2). Indeed, the maximal increase in left ventricular developed pressure elicited by isoproterenol at a dose of 10^{-6} M in WKY control rats and SHR was 127±14 and 69±14%, respectively ($P=0.01$). Nevertheless, the inotropic potency of isoproterenol, as assessed by pEC_{50} values, was not different in the two experimental groups (WKY rats=8.6±0.1 M, SHR=8.6±0.3 M).

Despite a profound inhibition of contractile responses to β-adrenoceptor activation, inotropic effects elicited by PDE inhibitors were preserved in SHR as compared to WKY control rats (Fig. 3). Both the magnitude of the maximal inotropic responses and the pEC_{50} values obtained following infusions of amrinone, milrinone and rolipram were

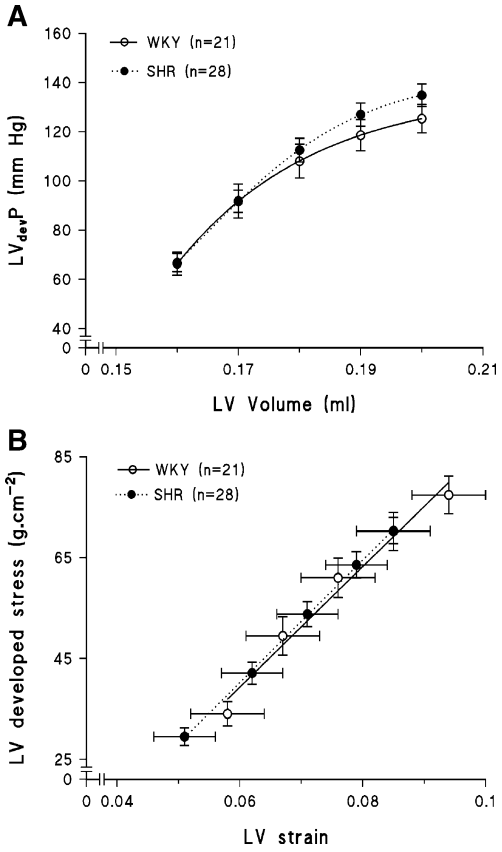

Fig. 1. A comparison of left ventricular systolic chamber (A) and myocardial function (B) as assessed ex vivo in spontaneously hypertensive and Wistar–Kyoto control rats. The slopes of the left ventricular (LV) developed pressure ($LV_{dev}P$)–volume and stress–strain relations were determined to assess ventricular systolic chamber and myocardial function, respectively. No differences between Wistar–Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) were noted (Table 1). Values in parenthesis reflect the number of experiments. Means±S.E.M.

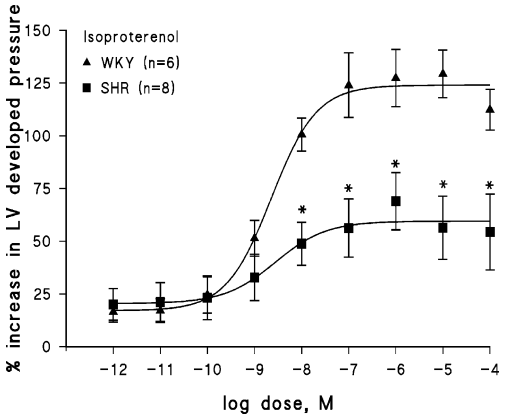

Fig. 2. A comparison of left ventricular inotropic responses elicited by isoproterenol in spontaneously hypertensive and Wistar–Kyoto control rats. The ordinate represents percent increase in left ventricular (LV) developed pressure in Wistar–Kyoto (WKY) rats and spontaneously hypertensive rats (SHR), and the abscissa the log of molar concentrations of isoproterenol. *$P<0.05$ versus WKY rats. Values in parenthesis reflect the number of individual determinations performed for each concentration of isoproterenol to reconstruct dose–response curves. Means±S.E.M.

similar in SHR as compared to WKY control rats (Table 3). With the exception of pentoxifylline, which was noted to be the most potent inotrope of the PDE inhibitors in SHR, there were otherwise no differences between the various agents in the magnitude of the maximal inotropic responses or the inotropic potency (pEC_{50} values) in either WKY rats or SHR (Table 3).

8-Br-cAMP, a cell-permeable and PDE-resistant cAMP analogue, produced dose-dependent positive inotropic responses in both WKY rats and SHR (Fig. 4). As compared to WKY control rats, the inotropic potency of 8-Br-cAMP was increased in SHR (pEC_{50}: SHR=6.2±0.1 M; WKY rats=5.4±0.1 M, $P=0.0004$). The maximal inotropic response to 8-Br-cAMP achieved at a dose of 10^{-4} M was nevertheless unchanged in SHR (49±8%) as compared to WKY controls (41±9%).

4. Discussion

The main finding of the present study is that despite profound reductions in inotropic responses to β-adrenoceptor activation, contractile effects produced by the PDE III inhibitors, amrinone and milrinone, the PDE IV inhibitor, rolipram, and the non-selective PDE I–IV inhibitor, pentoxifylline, as well as a cAMP analogue are preserved in cardiac hypertrophy in rats.

The mechanisms responsible for reduced inotropic responses elicited by β-adrenoceptor agonists in cardiac hypertrophy are likely to be through downregulation of myocardial β_1-adrenoceptors in SHR. Indeed, β_1-adreno-

Table 2
Baseline left ventricular developed pressures and +dP/dt values when assessing contractile responses to inotropes in spontaneously hypertensive and Wistar–Kyoto control rats

	Left ventricular developed pressure (mm Hg)		Left ventricular +dP/dt (mm Hg/s)	
	WKY rats	SHR	WKY rats	SHR
Isoproterenol	113±8 ($n=6$)	114±9 ($n=8$)	1935±199 ($n=6$)	1800±236 ($n=8$)
Amrinone	112±4 ($n=7$)	98±7 ($n=9$)	1853±93 ($n=7$)	1741±123 ($n=9$)
Milrinone	106±9 ($n=8$)	90±3 ($n=8$)	1809±95 ($n=8$)	1503±233 ($n=8$)
Rolipram	95±10 ($n=6$)	100±8 ($n=5$)	1770±278 ($n=6$)	1831±215 ($n=5$)
Pentoxifylline	92±6 ($n=8$)	96±10 ($n=8$)	1656±99 ($n=8$)	1714±80 ($n=8$)
8-Br-cAMP	105±10 ($n=7$)	102±6 ($n=11$)	1844±227 ($n=7$)	1718±117 ($n=11$)

No differences were noted between Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR).

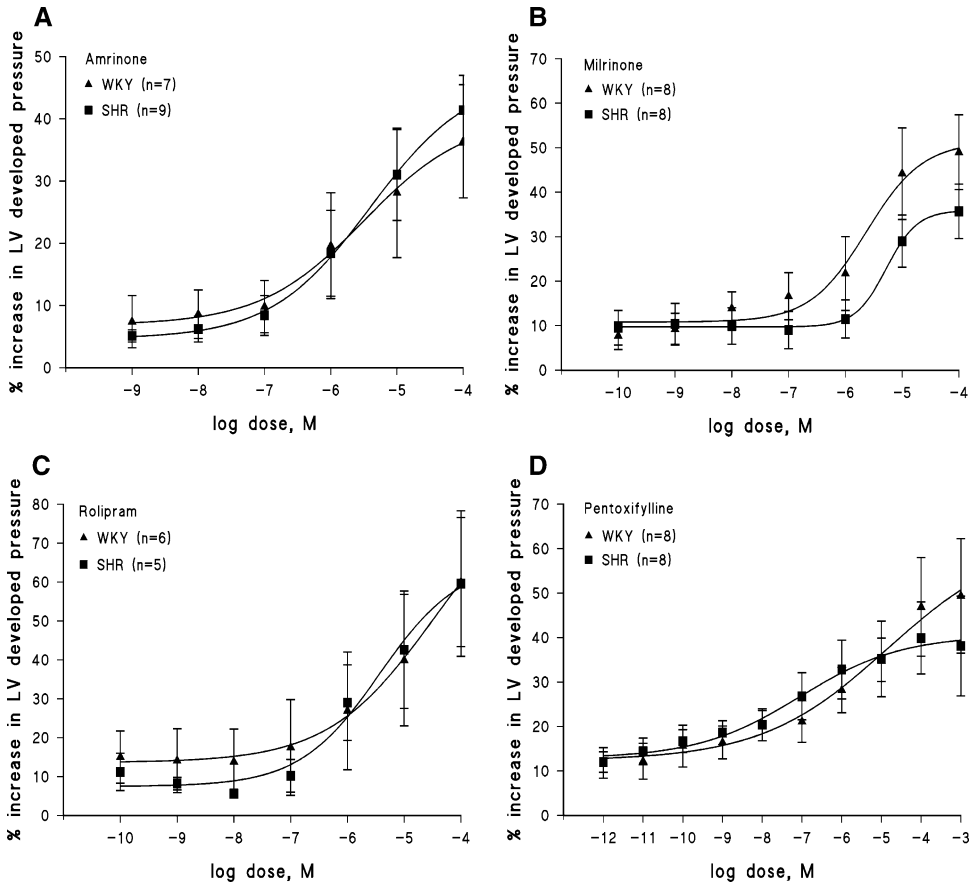

Fig. 3. A comparison of left ventricular inotropic responses elicited by the phosphodiesterase inhibitors in spontaneously hypertensive and Wistar–Kyoto control rats. Dose–response curves comparing the inotropic effects of amrinone (panel A), milrinone (panel B), rolipram (panel C), and pentoxifylline (panel D) in Wistar–Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) are shown. The ordinates indicate percent increase in left ventricular (LV) developed pressure, and the abscissa the log of molar concentrations of substances used. Values in parenthesis reflect the number of individual determinations performed for each concentration of phosphodiesterase inhibitors to reconstruct dose–response curves. No differences in contractile responses were noted at each concentration between spontaneously hypertensive and Wistar–Kyoto rats. pEC_{50} values for each agent are indicated in Table 3. Means±S.E.M.

ceptor mRNA levels and density were found to be reduced in cardiac muscle of SHR (Castellano et al., 1993; Bohm et al., 1988a, 1994). These alterations in β-adrenoceptor function are thought to be the result of profound sympathetic activation in SHR. Furthermore, reduced contractile responses to β-adrenoceptor agonists in SHR could also result from a diminished activation of myocardial adenylate cyclase (Amer et al., 1974; Ramanathan et al., 1976), an effect attributed to overexpression of $G_{i\alpha}$-proteins (Bohm et al., 1994).

The data reported on in the present study showing similar contractile effects of PDE inhibitors in SHR as compared to WKY rats are consistent with data obtained by Bohm et al. (1989) who demonstrated that in isolated rat papillary muscle inotropic effects elicited by milrinone were unchanged in SHR at 14–18 weeks of age. However, the

Table 3
A comparison of the inotropic potency (pEC_{50}) and efficacy (maximal inotropic response) of phosphodiesterase inhibitors in spontaneously hypertensive and Wistar–Kyoto control rats

	pEC_{50} values (M)		Maximal inotropic response[†] (%)	
	WKY rats	SHR	WKY rats	SHR
Amrinone	5.5±0.3 (n=7)	5.4±0.1 (n=9)	36±9 (n=7)	41±6 (n=9)
Milrinone	5.6±0.3 (n=8)	5.3±0.04 (n=8)	49±8 (n=8)	36±6 (n=8)
Rolipram	4.3±0.7 (n=6)	5.4±0.7 (n=5)	60±17 (n=6)	60±19 (n=5)
Pentoxifylline	5.0±0.7 (n=8)	7.1±0.3[a] (n=8)	47±11 (n=8)	40±8 (n=8)

[a] $P<0.05$ versus amrinone, milrinone and rolipram pEC50 values in SHR. The values in parenthesis reflect the number of experiments.
[†] Indicates % increase in left ventricular developed pressure at 10^{-4} M concentrations of agents in Wistar–Kyoto (WKY) rats and spontaneously hypertensive rats (SHR).

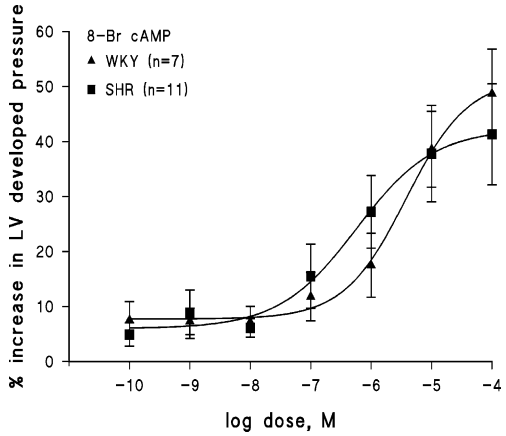

Fig. 4. A comparison of left ventricular inotropic responses elicited by 8-Br-cAMP in spontaneously hypertensive and Wistar–Kyoto control rats. The ordinate indicates percent increase in left ventricular (LV) developed pressure in Wistar–Kyoto (WKY) rats and spontaneously hypertensive rats (SHR), and the abscissa the log of molar concentrations of 8-Br-cAMP. Values in parenthesis reflect the number of individual determinations performed for each concentration of 8-Br-cAMP to reconstruct dose–response curves. No differences in contractile responses were noted at each concentration between spontaneously hypertensive and Wistar–Kyoto rats. Differences in the potency of 8-Br-cAMP between groups are indicated in the text. Means±S.E.M.

markedly low magnitude of milrinone-induced inotropic responses noted in normotensive rats of that age (Bohm et al., 1989), together with a lack of information regarding contractile effects elicited in response to inhibition of any other PDE isoenzymes found in the rat myocardium, makes the interpretation of these findings difficult. Indeed, chromatographic determination has revealed the presence of at least four PDE isoenzymes (PDE I–IV) in homogenates of rat cardiac muscle (Tenor et al., 1987; Shahid and Nicholson, 1990; Bode et al., 1991), of which two (PDE III and PDE IV) hydrolyze cAMP with a high affinity. Importantly, PDE isoforms interact in cAMP-mediated regulation of myocardial contractility. Indeed, inhibition of the PDE IV isoenzyme potentiates the stimulatory action of selective PDE III inhibitors on the L-type calcium current in isolated cardiomyocytes (Kajimoto et al., 1997), and sensitizes cardiac muscle to the inotropic influence of milrinone, a selective PDE III inhibitor (Shahid and Nicholson, 1990). In this regard, the strength of our present study is that we assessed the contractile effects elicited by inhibition of PDE III (amrinone and milrinone), PDE IV (rolipram), as well as by PDE I–IV (pentoxifylline) isoenzymes in isolated normal and hypertrophied rat heart muscle while excluding possible drug-induced variations in cardiac loads and heart rate.

Although a number of authors have reported on down-regulation of β-adrenergic signaling pathway in cardiac hypertrophy (Kumano et al., 1983; Bohm et al., 1988a, 1994; Castellano et al., 1993; Habuchi et al., 1995), the impact of heart disease on the activity of myocardial PDEs is unclear. Indeed, the rate of cAMP degradation by myocardial phosphodiesterases has been reported to be increased (Amer et al., 1974), decreased (Ramanathan et al., 1976) and unchanged (Klenerova et al., 1975; Hodgins and Frohlich, 1978; Sharma and Bhalla, 1978) in SHR. In addition, both decreases (Masunaga et al., 2004) and increases (Lee et al., 1994) in myocardial PDE activity were reported in cardiomyopathic hamsters. Furthermore, although a decreased milrinone-sensitive cAMP phosphodiesterase gene expression and activity were found in pacing-induced congestive heart failure in dogs (Smith et al., 1997), no changes in properties of PDE I–III isoenzymes occur in failing human heart preparations (Von der Leyen et al., 1991; Movsesian et al., 1991). In the present study the equivalent contractile responses to a variety of PDE inhibitors in SHR as compared to WKY rats suggests that either PDE enzyme activity remains unaltered in cardiac hypertrophy, or that any alterations are of little importance for the regulation of myocardial contraction.

In the present study, the pEC_{50} values of the PDE inhibitors assessed in WKY rats ranged from 4.3 to 5.6 M, values which are close to that for 8-Br-cAMP (5.4±0.1 M). Furthermore, the pEC_{50} values of the PDE inhibitors are consistent with the K_i values for these agents as determined from their ability to inhibit PDE isoenzyme activity in extracts of rat cardiac muscle (Earl et al., 1986; Tenor et al., 1987; Shahid and Nicholson, 1990; Meskini et al., 1994). In isolated heart preparations, the pEC_{50} values, as well as the magnitude of inotropic responses elicited by these agents in normotensive WKY rats, were apparently independent of the PDE isoenzyme (PDE III or PDE IV, PDE I–IV) inhibited. Similar effects were found in SHR, except that the inotropic potency of pentoxifylline, the non-selective PDE inhibitor, was greater than for rolipram, amrinone and milrinone. Whether this finding is explained by more effective cAMP generation following concomitant inhibition of myocardial PDE I–IV isoenzymes in SHR, remains to be established.

In the present study the increased potency of a cAMP analogue at eliciting contractile responses in SHR suggests enhanced signaling downstream from cAMP. Presently, it is unclear whether signaling pathways downstream from cAMP are also modified in cardiac hypertrophy. For instance, cardiac cAMP-dependent protein kinase activity was shown to be reduced in SHR (Prashad, 1985), which presumably could influence the extent of phosphorylation of cardiac regulatory proteins. However, increased phosphorylation of troponin I by cAMP-dependent protein kinase (McConnell et al., 1997) and increased stimulation of L-type calcium currents by cAMP (Xiao and McArdle, 1994) in SHR would suggest enhanced downstream signaling. Furthermore, concomitant administration of isoproterenol with PDE inhibitors restores reduced β-adrenoceptor-mediated effects on L-type calcium currents in cardiac myocytes from SHR (Habuchi et al., 1995), thus demonstrating preserved cAMP effects in hypertrophied myocardium.

In conclusion, the results of the present study provide clear evidence that despite a marked attenuation of

contractile responses to a β-adrenoceptor agonist in SHR with cardiac hypertrophy, inotropic responses to PDE inhibitors are preserved. These data suggest that unlike in chronic heart failure where downregulation of the β-adrenoceptor-cAMP pathway results in reduced inotropic responses to both β-adrenoceptor agonists and PDE inhibitors (Bohm et al., 1988b; Feldman et al., 1987; Von der Leyen et al., 1991), in cardiac hypertrophy downregulation of the same pathway only limits β-adrenoceptor agonist-induced contractile effects. Whether these findings translate into more appropriate therapeutic benefits (inotropic support) when utilizing PDE inhibitors in patients with cardiac hypertrophy and acute cardiac decompensation requires further investigation.

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