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Functional analysis of desensitization of the β-adrenoceptor signalling pathway in rat cardiac tissues following chronic isoprenaline infusion

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1 This study examined β-adrenoceptor signalling in cardiac tissues following infusion of isoprenaline (400 μg kg^{-1} h^{-1}) or vehicle to rats for 14 days.

2 Isoprenaline infusion caused marked hypertrophy of atria and ventricles and reduced the resting rate of spontaneously beating right atria and the basal force of left atrial contraction.

3 In spontaneously beating right atria, concentration-response curves to isoprenaline and forskolin were shifted 7.9 and 3.2 fold to the right following treatment whereas responses to the cyclic AMP analogue 5,6-dichloro-1-ß-D-ribofuranosylbenzimidazole-3′, 5′-cyclic monophosphorothioate were unchanged.

4 In electrically driven left atria, concentration-response curves to isoprenaline and forskolin were shifted 4 fold to the right and maximum responses reduced. Responses to dibutyryl cyclic AMP were shifted 3.2 fold to the right but those to Ca^{2+} were unchanged.

5 Inotropic responses of left and right ventricular papillary muscles to isoprenaline were abolished and markedly reduced respectively by isoprenaline treatment. Responses to forskolin were shifted 5 fold to the right. Responses to dibutyryl cyclic AMP were shifted to the right 3.2 and 2 fold in left and right ventricular papillary muscles. Responses to isobutyl methyl xanthine were shifted to the right 15.8 and 6.3 fold in left and right papillary muscles whereas those to Ca^{2+} were not significantly altered.

6 This study indicates differences in β-adrenoceptor desensitization in different regions of the heart following chronic infusion of isoprenaline. Chronotropic responses showed impaired signalling between the receptor and adenylate cyclase whereas inotropic responses exhibited additional desensitization at the level of cyclic AMP dependent protein kinase.

Keywords: β-adrenoceptors; cyclic AMP; cardiac hypertrophy; desensitization; forskolin; heart; isoprenaline

Abbreviations: AR, adrenoceptor; 5,6-DCl-cBIMP, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole-3′,5′-cyclicmonophosphorothioate; dibutyryl cyclic AMP, N^6,2′-O-dibutyryladenosine-3′5′-monophosphate; IBMX, 3-isobutyl-1-methylxanthine

Introduction

Long-term treatment of guinea-pigs and rats with isoprenaline results in cardiac changes which include hypertrophy (Stanton *et al*., 1969; Tse *et al*., 1979; Chang *et al*., 1982; Matthews *et al*., 1996) and desensitization of responses to β-adrenoceptor (AR) agonists (Tse *et al*., 1979; Nomura *et al*., 1982; Hayes *et al*., 1986; Martin & Broadley, 1994; Russell *et al*., 1994). Many studies have explored the mechanisms of β-AR desensitization operating at the level of the receptor. These include phosphorylation of the β-AR by cyclic AMP-dependent protein kinase and G-protein receptor kinases, sequestration and internalization of the phosphorylated receptor by β-arrestin followed over a longer time course by receptor down-regulation (reviewed in Summers *et al*., 1997). A large number of radioligand binding and autoradiographic studies have also indicated that treatment of animals with isoprenaline results in differential regulation of β_1- and β_2-ARs in the heart with β_1-ARs being far less susceptible to down-regulation than β_2-ARs (Nanoff *et al*., 1989; Lu & Barnett, 1990; Molenaar *et al*., 1990; Kompa *et al*., 1994; Matthews *et al*., 1996).

Inotropic and chronotropic responses of the heart are mediated predominantly in most species, and almost exclusively in rat, by β_1-ARs (Juberg *et al*., 1985; Xiao & Lakatta, 1993). Cardiac preparations exhibit a large β_1-AR reserve (Kaumann, 1978; Venter, 1979), isoprenaline needing to occupy less than 20% of rat atrial or ventricular papillary muscle β_1-ARs to produce a 90% maximal inotropic response (Doggrell, 1990; Brown *et al*., 1992). The maximal reductions in cardiac β_1-ARs of animals treated with isoprenaline or noradrenaline are 40–50% (Nanoff *et al*., 1989; Molenaar *et al*., 1990; Kompa *et al*., 1994; Matthews *et al*., 1996; Zhao *et al*., 1996) which are therefore unlikely to fully account for the marked reduction of β_1-AR responsiveness observed in these desensitization models (Tse *et al*., 1979; Nomura *et al*., 1982; Hayes *et al*., 1986; Russell *et al*., 1994). Indeed, a 7 day infusion of isoprenaline to guinea-pigs caused a marked rightward shift of the atrial concentration-response curve to isoprenaline and decrease in the maximal inotropic response without alteration to the density of β_1-ARs (Russell *et al*., 1994). Furthermore, ventricular myocytes isolated from rats infused with noradrenaline for 2 weeks displayed impaired contractile responses not only to isoprenaline but also to forskolin and dibutyryl cyclic AMP, indicating alterations to post-receptor signalling (Jones *et al*., 1990).

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We therefore examined β-AR signalling in the heart following infusion of rats with isoprenaline (400 μg kg^{-1} h^{-1}) or vehicle for 2 weeks. Since the extent of β-AR desensitization differs between atrial and ventricular preparations (Chess-Williams, 1993; Butterfield & Chess-Williams, 1993) and between inotropic and chronotropic responses (Kaumann & Birnbaumer, 1976; Ball *et al*., 1982; Herepath & Broadley, 1990; Butterfield & Chess-Williams, 1993; Russell *et al*., 1994), we examined chronotropic responses of spontaneously beating right atria and the inotropic responses of electrically driven left atria and left and right ventricular papillary muscles to agents acting at several points of the β-AR-cyclic AMP signalling pathway.

Methods

Subcutaneous infusion of isoprenaline

Rats (male Sprague-Dawley, 230–260 g) were anaesthetized with methohexitone sodium (60 mg kg^{-1} i.p.) and a small region at the back of the neck shaved and cleaned with 1% Hibitane. A small incision was made and the osmotic minipump (Alzet model 2002) subcutaneously implanted. The wound was closed with a Michel clip and sprayed with OpSite™ (Smith and Nephew, Hull, U.K.). The pumps infused isoprenaline HCl at 400 μg kg^{-1} h^{-1} or vehicle (1 mM HCl) for 14 days. The rats were maintained on a 12 h light/dark cycle at 22°C with free access to food and water.

After pretreatment, rats were rendered unconscious with 80% CO_2/20% O_2 and exsanguinated. The right and left atria and the left and right ventricular papillary muscles were rapidly removed and placed in Krebs buffer (composition in mM); NaCl 119.8, KCl 5.4, $CaCl_2$ 1.8, $NaHCO_3$ 22.6, $MgSO_4$ 1.05, NaH_2PO_4 0.42, glucose 5.5, EDTA 0.05, ascorbate 0.28, gassed with 95% O_2/5% CO_2. Tissues were set up in 5 ml organ baths under 5 mN (atria) or 10 mN (papillary muscles) tension. Responses were recorded isometrically using Grass FTO3 transducers and a MacLab recording system. Right atria were allowed to beat spontaneously whereas left atrium, left papillary muscle and right papillary muscle were electrically driven at supramaximal voltage at 5 and 1 Hz with 2 and 5 msec pulses respectively. While overt necrotic damage of atria and ventricular papillary muscles was not readily apparent following isoprenaline treatment of animals, in line with previous studies using similar doses of isoprenaline (Tse *et al*., 1979; Matthews *et al*., 1996), a small number of papillary muscles which showed macroscopic signs of scarring (patches of discolouration) were discarded.

Tissues were allowed to equilibrate for 45 min during which time the bathing solution was changed every 10 min. Cumulative concentration-response curves were carried out to calcium chloride; N^6,2′-O-dibutyryladenosine-3′5′-monophosphate (dibutyryl cyclic AMP, in left atrium, left papillary muscle and right papillary muscle); 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole-3′,5′-cyclic monophosphorothioate (5,6-DCl-cBIMP, in right atrium); forskolin; 3-isobutyl-1-methylxanthine (IBMX, in left papillary muscle and right papillary muscle) and isoprenaline HCl. One concentration-response curve was carried out in each tissue. At the end of each experiment, tissues were blotted and weighed.

Calculations

Results for spontaneously beating right atrium are expressed as changed rate of beating in response to each agent. Results for left atrium, left papillary muscle and right papillary muscle inotropic responses are expressed as a change in force in mN g^{-1} wet tissue weight. All basal values are given in Results. All experimental values are given as a mean ± s.e.mean. Maximum values and pD_2 values were calculated using the computer software PRISM (GraphPad Inc., San Diego, U.S.A.) and analysed by an unpaired Student's *t*-test with a probability of less than 0.05 considered significant.

Table 1 Body, heart and left (LV) and right (RV) ventricular wet weights of vehicle- and isoprenaline-treated rats

	Body wt (g)	*Heart wt* (g)	*Heart/body wt ratio* (mg g^{-1})	*LV wet wt* (g)	*LV/body wt ratio* (mg g^{-1})	*RV wet wt* (g)	*RV/body wt ratio* (mg g^{-1})	n
Vehicle	362.1 ± 4.0	1.49 ± 0.03	4.08 ± 0.08	0.42 ± 0.01	1.15 ± 0.02	0.17 ± 0.00	0.46 ± 0.01	54
Isoprenaline	371.2 ± 2.6	2.12 ± 0.03	5.71 ± 0.09	0.54 ± 0.01	1.46 ± 0.02	0.22 ± 0.00	0.59 ± 0.01	53
	$P = 0.06$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	

Values are mean ± s.e.mean and *n* represents the number of animals.

Table 2 Wet weight and basal rate of spontaneously beating right atria or basal force of electrically driven left atria and left and right ventricular papillary muscles from vehicle- and isoprenaline-treated rats

Tissue	*Treatment*	*Wet weight* (mg)	*Basal rate (b.p.m.) or force* (mN g^{-1} tissue wt)	n
Right atrium	Vehicle	60.7 ± 3.5	284 ± 8	22
	Isoprenaline	88.4 ± 5.3	240 ± 6	24
		$P < 0.0001$	$P < 0.0001$	
Left atrium	Vehicle	29.6 ± 1.3	35 ± 3	31
	Isoprenaline	59.6 ± 1.5	15 ± 1	27
		$P < 0.0001$	$P < 0.0001$	
Left papillary muscle	Vehicle	7.6 ± 0.5	833 ± 58	37
	Isoprenaline	8.4 ± 0.4	712 ± 68	37
		$P = 0.25$	$P = 0.18$	
Right papillary muscle	Vehicle	5.0 ± 0.3	917 ± 96	34
	Isoprenaline	6.1 ± 0.3	753 ± 77	33
		$P = 0.01$	$P = 0.19$	

Values are mean ± s.e.mean and *n* represents the number of animals.

Materials

The following drugs were used: N^6,2′-O-dibutyryladenosine-3′,5′-monophosphate (dibutyryl cyclic AMP), forskolin, 3-isobutyl-1-methylxanthine (IBMX), (−)-isoprenaline hydrochloride (Sigma, St. Louis, U.S.A.); 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole-3′,5′-cyclic monophosphorothioate (5,6-DCl-cBIMP, BIOLOG, Bremen, Germany); sodium methohexitone (Eli Lilly, West Ryde, Australia). Stock solutions of dibutyryl cyclic AMP (200 mM), IBMX (5 mM) and isoprenaline (10 mM) were made using distilled water. Forskolin and 5, 6-DCl-cBIMP (both 10 mM) were dissolved in dimethylsulphoxide and stored as aliquots at −20°C until use.

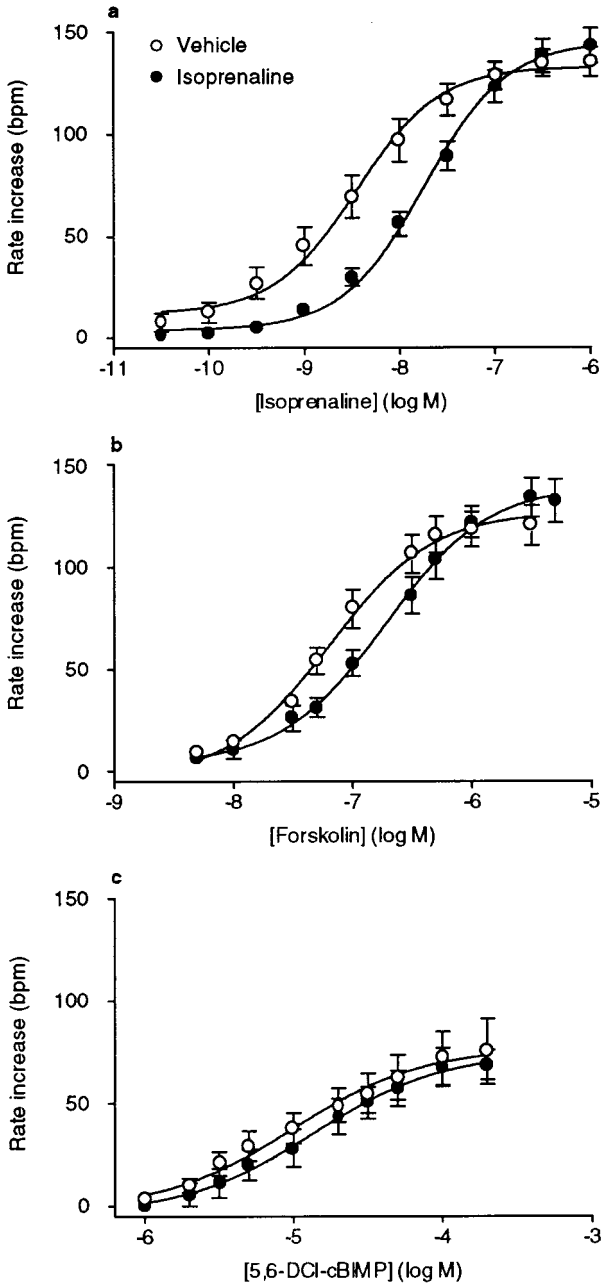

Figure 1 Mean chronotropic responses of spontaneously beating right atrium from vehicle and isoprenaline-treated rats to cumulative additions of isoprenaline (a), forskolin (b) and 5,6-DCl-cBIMP (c). Points show mean values and vertical lines indicate s.e.mean ($n=5-9$).

Results

Isoprenaline infusion causes cardiac hypertrophy

Infusion of isoprenaline by osmotic mini pumps subcutaneously for 14 days caused marked cardiac hypertrophy. Heart weight was increased 42.3%, heart weight/body weight ratio by 40.0%, left ventricular wet weight by 28.6%, left ventricle/body weight ratio by 27% and right ventricular wet weight by 29.4% (Table 1). Significant hypertrophy also occurred in right atrium, left atrium and right ventricular papillary muscle (Table 2).

Effects of isoprenaline infusion on basal rate and force of atrial and ventricular tissues

In rat isolated spontaneously beating right atrium, the resting rate was decreased from 284±8 b.p.m. in control to 240±6 b.p.m. in isoprenaline treated animals (Table 2). In electrically driven left atrium the basal force of contraction was decreased from 35±3 to 15±1 mNg^{-1} tissue weight. The basal force of contraction of the ventricular papillary muscles tended to decrease, although not to a statistically significant level (Table 2).

Effects of isoprenaline infusion on chronotropic responses of right atrium

In spontaneously beating right atrium, the concentration-response curve to isoprenaline was shifted 7.9 fold to the right following isoprenaline infusion ($P<0.05$, $n=7$) with no significant change in the maximum response (Figure 1a and Table 3). Concentration-response curves to the direct activator of adenylate cyclase, forskolin, were also shifted 3.2 fold parallel to the right by isoprenaline infusion (Figure 1b and Table 3). Concentration-response curves to the membrane permeant cyclic AMP analogue dibutyryl cyclic AMP could not be carried out in the isolated spontaneously beating right atrium because the atrium ceased to beat regularly at relatively low concentrations of dibutyryl cyclic AMP, probably due to direct depressant effects of butyrate on the pacemaker cells. Instead, concentration-dependent chronotropic responses to another membrane permeant cyclic AMP analogue 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole-3′, 5′-cyclic monophosphorothioate (5,6-DCl-cBIMP, Sandberg *et al.*, 1991; Schultz *et al.*, 1994) were

Table 3 pD_2 values and maximal chronotropic responses of spontaneously beating right atria from vehicle- and isoprenaline-treated rats to isoprenaline, forskolin and the cell permeable cyclic AMP analogue 5,6-DCl cBIMP

	pD_2 values		
	Agonist		
Animal treatment	*Isoprenaline*	*Forskolin*	*5,6-DCl cBIMP*
Vehicle	8.7±0.1 (9)	7.2±0.0 (8)	5.0±0.2 (5)
Isoprenaline	7.8±0.1 (9)	6.7±0.1 (8)	4.8±0.2 (6)
	$P=0.002$	$P=0.002$	$P=0.57$
	Maximal responses (Δ b.p.m.)		
	Isoprenaline	*Forskolin*	*5,6-DCl cBIMP*
Vehicle	134±7 (9)	123±8 (8)	77±16 (5)
Isoprenaline	143±9 (9)	133±10 (8)	72±7 (6)
	$P=0.45$	$P=0.40$	$P=0.77$

Values are mean±s.e.mean and *n* represents the number of animals.

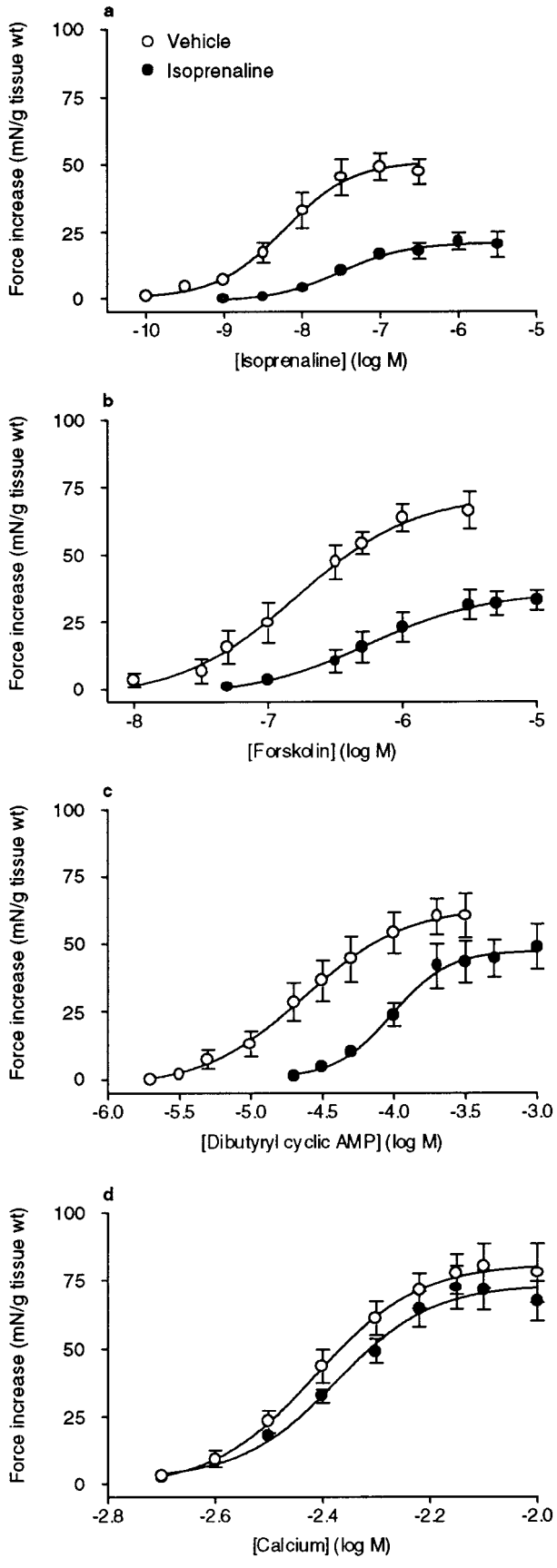

Figure 2 Mean inotropic responses of electrically driven left atrium from vehicle and isoprenaline-treated rats to cumulative additions of isoprenaline (a), forskolin (b), dibutyryl cyclic AMP (c), and calcium chloride (d). Points show mean values and vertical lines indicate s.e.mean ($n = 5-10$).

examined. There was no significant difference between responses to 5,6-DCl-cBIMP in right atria from isoprenaline-infused and vehicle-infused animals (Figure 1c and Table 3).

Effects of isoprenaline infusion on inotropic responses of electrically driven left atria

In rat isolated electrically driven left atrium, the concentration response curves to isoprenaline were shifted in isoprenaline infused rats 4 fold to the right together with a marked 57% decrease in the maximum response (Figure 2a and Table 4). Concentration response curves to forskolin showed a similar pattern being also shifted to the right 4 fold together with a 52% decrease in maximal response (Figure 2b and Table 4). Concentration response curves to the membrane permeant cyclic AMP analogue dibutyryl cyclic AMP were shifted 3.2 fold in parallel to the right with no significant change in the maximal response (Figure 2c and Table 4). Ca^{2+} was used to examine whether isoprenaline treatment had any effects on the contractile machinery of the left atrium. Concentration-response curves to Ca^{2+} showed no significant shift or change in the maximal response (Figure 2d and Table 4). The inotropic responses in left atrium therefore showed desensitization that was much more marked than that observed for the chronotropic responses in right atrium and was associated with decreases in maximal responses to both isoprenaline and forskolin. There was also evidence of desensitization to direct activation of cyclic AMP-dependent protein kinase but no evidence of direct desensitization of the contractile machinery.

Effect of isoprenaline treatment on inotropic responses of electrically driven left and right ventricular papillary muscles

The pattern of desensitization seen for the inotropic response in left ventricular papillary muscle and right ventricular papillary muscle was similar except for greater depression of maximal responses observed in the left papillary muscle. Concentration response curves to isoprenaline were obtained in both left and right papillary muscles of animals that had been infused with vehicle. However, following isoprenaline infusion, the response to isoprenaline was totally abolished in left papillary muscle (Figure 3a) and very markedly reduced in right papillary muscle (Figure 4a). Responses were however obtainable to forskolin in isoprenaline treated animals, although these were markedly shifted to the right without major changes in the maximal response. In left and right papillary muscles the concentration-response curves to forskolin were shifted 5 fold to the right (Figures 3b, 4b and Table 5) with no significant change in the maximal response. A similar pattern was seen with the concentration-response curves to dibutyryl cyclic AMP which were shifted in parallel to the right 3.2 and 2 fold respectively in left and right papillary muscles with a significant 42% reduction in the maximal response in left papillary muscle (Figures 3c, 4c and Table 5). Responses were also examined to the inhibitor of phosphodiesterase, IBMX, which produced concentration-dependent positive inotropic responses which were shifted to the right 15.8 and 6.3 fold in left and right papillary muscles respectively in the isoprenaline pretreated animals (Figures 3d, 4d and Table 5). There were no significant changes in the maximal

Table 4 pD_2 values and maximal force responses of electrically driven left atria from vehicle- and isoprenaline-treated rats to isoprenaline, forskolin, dibutyryl cyclic AMP and calcium chloride

		pD_2 values *Agonist*		
Animal treatment	*Isoprenaline*	*Forskolin*	*DbcAMP*	*$CaCl_2$*
Vehicle	8.2±0.1 (6)	6.8±0.2 (6)	4.5±0.1 (10)	2.4±0.0 (9)
Isoprenaline	7.6±0.1 (5)	6.2±0.1 (5)	4.0±0.1 (10)	2.4±0.0 (8)
	$P<0.001$	$P=0.022$	$P<0.001$	$P=0.16$
		Maximal force responses (Δ mN g^{-1} tissue wt)		
	Isoprenaline	*Forskolin*	*DbcAMP*	*$CaCl_2$*
Vehicle	49±5 (6)	66±7 (6)	63±6 (10)	80±8 (9)
Isoprenaline	21±3 (5)	32±4 (5)	45±7 (10)	72±8 (8)
	$P=0.002$	$P=0.003$	$P=0.064$	$P=0.5$

Values are means±s.e.mean and *n* represents the number of animals.

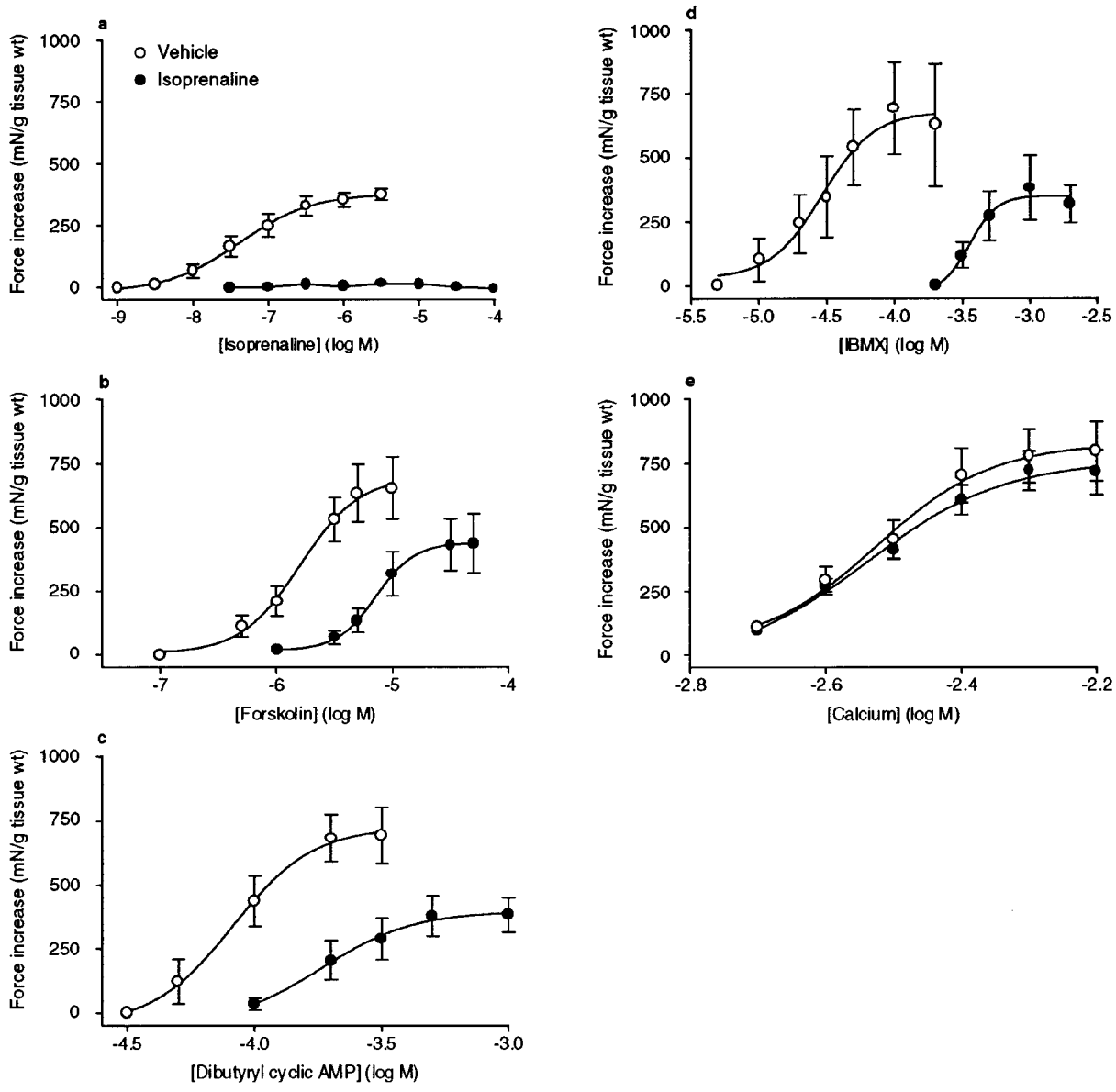

Figure 3 Mean inotropic responses of electrically driven left ventricular papillary muscle from vehicle and isoprenaline-treated rats to cumulative additions of isoprenaline (a), forskolin (b), dibutyryl cyclic AMP (c), IBMX (d) and calcium chloride (e). Points show mean values and vertical lines indicate s.e.mean ($n=4-12$).

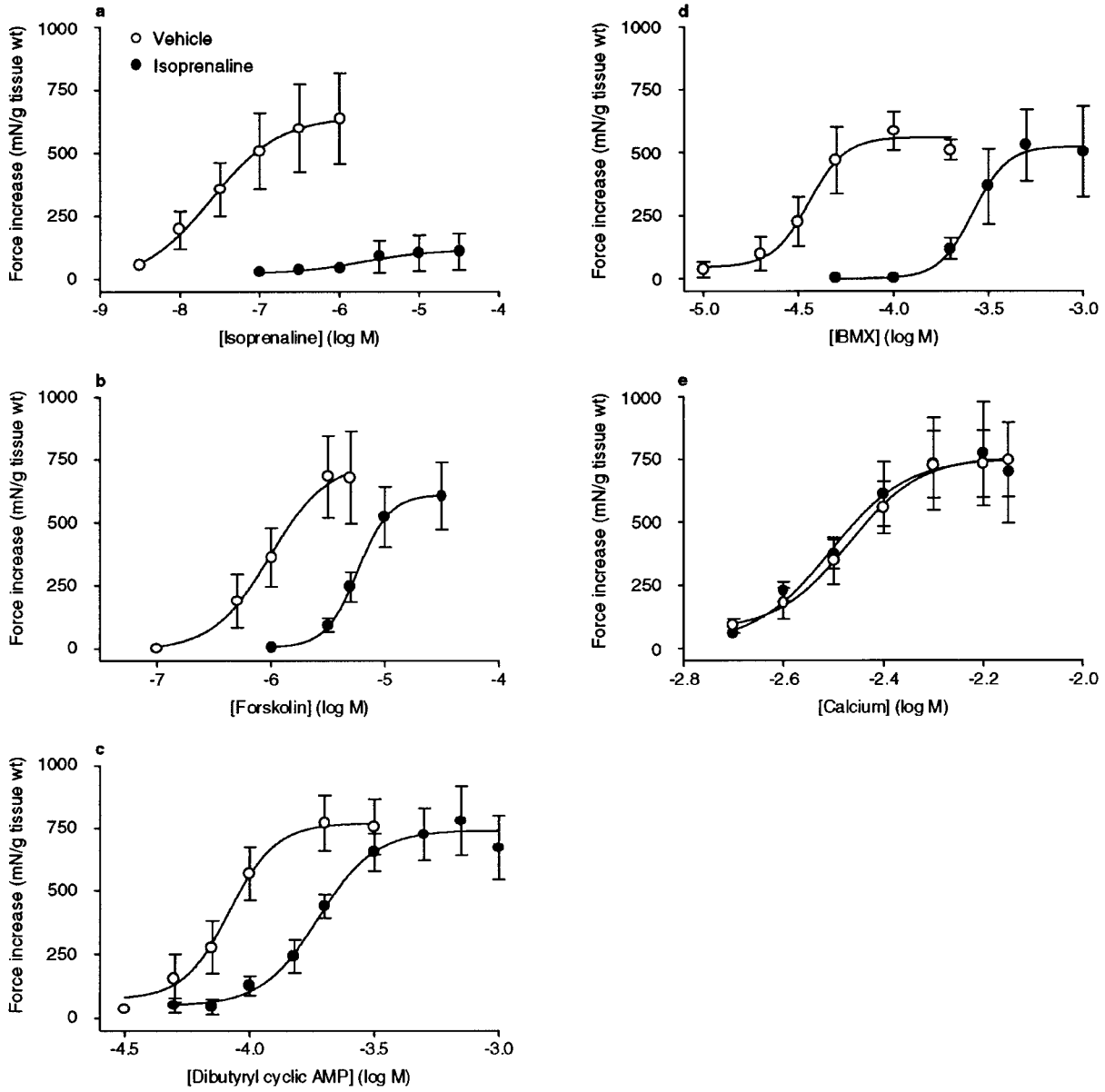

Figure 4 Mean inotropic responses of electrically driven right ventricular papillary muscle from vehicle and isoprenaline-treated rats to cumulative additions of isoprenaline (a), forskolin (b), dibutyryl cyclic AMP (c), IBMX (d) and calcium chloride (e). Points show mean values and vertical lines indicate s.e.mean ($n=4-9$).

responses to IBMX in either tissue. In both preparations, responses to direct activation of the contractile machinery by Ca^{2+} were not significantly altered by isoprenaline treatment (Figures 3e, 4e and Table 5).

Discussion

We have examined changes in the β-AR-cyclic AMP signalling pathway in hearts of rats chronically infused with isoprenaline. Desensitization of β-adrenoceptor-mediated responses were associated with impaired signalling at a number of points in the signal transduction pathway and the pattern of desensitization differed between chronotropic responses of the spontaneously beating right atrium and the inotropic responses of left atrium and ventricular papillary muscles. Differences were also observed between the desensitization of left atrial inotropic responses and ventricular inotropic responses.

In right atrium, concentration-response curves for chronotropic responses to isoprenaline were shifted to the right with EC_{50} values 8 fold higher for isoprenaline-treated animals compared to vehicle-treated controls, without alteration of the maximal response. Previous studies have shown unchanged chronotropic responses of right atrium to isoprenaline following infusion of rats with isoprenaline (40 $\mu g\ kg^{-1}\ h^{-1}$ for 3 days, Butterfield & Chess-Williams 1993), a 3.2 fold shift to the right of the concentration-response curve and reduction in maximum (40 $\mu g\ kg^{-1}\ h^{-1}$ for 7 days, Martin & Broadley, 1994) or a 13.2 fold shift to the right and no change in the maximal response (400 $\mu g\ kg^{-1}\ h^{-1}$ for 7 days, Chang *et al.*, 1982). An autoradiographic study showed that the β-AR density of the sinoatrial node in the same rat model of desensitization was reduced by 50% following isoprenaline infusion (Matthews *et al.*, 1996). A significantly reduced responsiveness to forskolin (a 3.2 fold shift to the right) reported here indicates that although down-regulation of β-

Table 5 pD_2 values and maximal force responses of electrically driven left and right ventricular papillary muscles from vehicle- and isoprenaline-treated rats to isoprenaline, forskolin, dibutyryl cyclic AMP, IBMX and calcium chloride

	pD_2 values left ventricular papillary muscle				
			Agonist		
Animal treatment	*Isoprenaline*	*Forskolin*	*DbcAMP*	*IBMX*	*$CaCl_2$*
Vehicle	7.4±0.3 (4)	5.8±0.1 (9)	4.1±0.1 (8)	4.4±0.2 (5)	2.6±0.0 (11)
Isoprenaline	– (6)	5.1±0.1 (9)	3.6±0.1 (8)	3.2±0.2 (6)	2.6±0.0 (12)
		$P=0.0011$	$P<0.0001$	$P=0.0011$	$P=0.86$
	pD_2 values right ventricular papillary muscle				
	Isoprenaline	*Forskolin*	*DbcAMP*	*IBMX*	*$CaCl_2$*
Vehicle	7.6±0.1 (6)	6.0±0.1 (7)	4.1±0.0 (8)	4.4±0.1 (5)	2.5±0.0 (9)
Isoprenaline	– (6)	5.3±0.1 (6)	3.8±0.0 (9)	3.6±0.1 (4)	2.5±0.0 (8)
		$P<0.0001$	$P=0.0002$	$P<0.0001$	$P=0.41$
	Maximal responses (ΔmN g^{-1} tissue weight) *left ventricular papillary muscle*				
	Isoprenaline	*Forskolin*	*DbcAMP*	*IBMX*	*$CaCl_2$*
Vehicle	376±24 (4)	606±117 (9)	659±102 (8)	590±174 (5)	774±103 (11)
Isoprenaline	13±6 (6)	432±100 (9)	381±67 (8)	383±126 (4)	714±88 (12)
	$P<0.0001$	$P=0.28$	$P=0.039$	$P=0.35$	$P=0.66$
	Maximal responses (Δ mN g^{-1} tissue weight) *right ventricular papillary muscle*				
	Isoprenaline	*Forskolin*	*DbcAMP*	*IBMX*	*$CaCl_2$*
Vehicle	637±180 (6)	679±184 (7)	754±111 (8)	585±78 (5)	747±14 (9)
Isoprenaline	108±73 (6)	605±134 (6)	724±104 (9)	528±142 (4)	695±200 (8)
	$P=0.021$	$P=0.76$	$P=0.84$	$P=0.72$	$P=0.84$

Values are mean±s.e.mean and *n* represents the number of animals.

ARs almost certainly contributes to the desensitization, changes more distal in the signalling pathway are also involved. Although forskolin interacts directly with the catalytic subunit of adenylate cyclase, this interaction is influenced by the guanine nucleotides Gsα and Giα (Seamon & Daly, 1986) and alterations in responses to forskolin could therefore indicate an impairment at the level of adenylate cyclase or alterations to Gs or Gi. An absence of change in reponses to the membrane-permeant cyclic AMP analogue 5, 6-DCl-cBIMP following chronic isoprenaline suggests that desensitization of right atrial rate responses is associated with alterations located between the receptor and adenylate cyclase and do not extend to cyclic AMP-dependent protein kinase.

Inotropic responses of left atrium showed a different pattern of desensitization from that of the chronotropic responses of right atrium. Concentration-response curves for isoprenaline and forskolin both indicated desensitization with 4 fold shifts to the right associated with marked reductions in maximal responses (58% reduction for isoprenaline and 52% for forskolin). Previous studies of left atrial inotropic responses to isoprenaline following infusion of rats with isoprenaline have also indicated marked reductions in responsiveness to isoprenaline (Chang *et al*., 1982; Butterfield & Chess-Williams 1993; Kenakin & Ferris, 1983; Martin & Broadley, 1994). A similar reduction in responsiveness to isoprenaline and forskolin implies that alterations at the level of β-ARs are unlikely to contribute greatly to the desensitization seen in left atrium. Indeed, although atrial β_1-ARs were reduced by 46% in this rat model of desensitization (Matthews *et al*., 1996), left atrial responses to β-AR stimulation have been shown to have a large receptor reserve, with an occupation of $<20\%$ of β-ARs by isoprenaline sufficient to produce a 90–100% maximum response (Doggrell, 1990; Brown *et al*., 1992). In addition to the reduced responsiveness of left atrium to forskolin, there was also an attenuation of responses to dibutyryl cyclic AMP, with a 3.2 fold shift to the right of the concentration-response curve and a tendency for suppression of the maximum ($P=0.06$). These findings indicate that desensitization of left atrial β-AR responsiveness is likely to involve impairment at a number of points in the post-receptor signalling pathway including G proteins, adenylate cyclase, cyclic AMP-dependent protein kinase or beyond. The absence of change in responses to Ca^{2+} indicated that atrial contractile function was not impaired.

Differences in the pattern of β-AR desensitization for inotropic and chronotropic responses of atria have previously been reported. Isoprenaline, both *in vitro* (Kaumann & Birnbaumer, 1976; Herepath & Broadley, 1990) and *in vivo* (Butterfield & Chess-Williams, 1993; Russell *et al*., 1994), induced a more marked desensitization of inotropic responses to β-AR stimulation than chronotropic responses. The reasons for this are unclear. Russell *et al*. (1994) reported that in the guinea-pig, 7 days infusion of isoprenaline (400 μg kg^{-1} h^{-1}) did not result in a change to β_1-AR density in the atrial myocardium whereas a 34% reduction was observed in the sinoatrial node. Such an anatomical difference in β_1-AR down-regulation was not observed for rats infused with isoprenaline for 2 weeks (Matthews *et al*., 1996), with both the sinoatrial node and atrial myocardium undergoing a similar loss of β_1-ARs (50 and 46% respectively). We have shown, however, that in addition to the desensitization of inotropic responses being associated with a markedly reduced maximal response to isoprenaline, unlike that of the chronotropic responses of right atrium, the inotropic responses of left atrium to the cyclic AMP analogue dibutyryl cyclic AMP were impaired while the chronotropic responses of the right atrium to another cyclic AMP analogue, 5,6-DCl-cBIMP, were unaffected by chronic isoprenaline. Unfortunately, right atrial chronotropic responses to dibutyryl cyclic AMP could not be examined due to the toxic effects of that analogue, or its metabolites, on the rate response. While it cannot be ruled out that the difference in desensitization observed for inotropic and chronotropic responses was due to differences in the two cyclic AMP analogues used, rather than to true tissue differences, the specificity of 5,6-DCl-cBIMP for cyclic AMP dependent

protein kinase and a correlation between the functional consequences of addition of cyclic AMP to membranes or intact cell preparations and that of 5,6-DCl-cBIMP have been established (Sandberg *et al*., 1991; Schultz *et al*., 1994). It would of course be useful to examine inotropic responses to 5,6-DCl-cBIMP to confirm the differences in the pattern of desensitization at the level of cyclic AMP dependent protein kinase between inotropic and chronotropic responses.

Desensitization of inotropic responses of ventricular tissues (right and left papillary muscles) resembled that seen in left atrium. The desensitization was pronounced and was associated with reduced responsiveness to isoprenaline, forskolin and dibutyryl cyclic AMP. As in the left atrium there was no significant change in responses to Ca^{2+}. However, unlike left atrium, there was a total abolition of responses to isoprenaline in left papillary muscle of isoprenaline-treated animals and an almost complete abolition of responses of right papillary muscle. These findings support the notion that ventricular function is more sensitive to a loss of β-ARs than left atrium, in accord with previous studies (Butterfield & Chess-Williams, 1993; Chess-Williams, 1993), and indicates that atrial tissues may have a larger β_1-AR reserve than ventricular tissues. Since the ventricles have a higher density of β_1-AR binding sites than atrium (Chess-Williams *et al*., 1987; Matthews *et al*., 1996), coupling of β_1-ARs to the cyclic AMP signalling pathway appears to be more efficient in the atrium.

Inotropic responses of left and right ventricular papillary muscles to forskolin were attenuated with a 5 fold shift to the right of the concentration-response. However, unlike the responses to isoprenaline or the situation in left atrium, the maximal responses of ventricular papillary muscles to forskolin were only slightly reduced in left papillary muscle and unchanged in right papillary muscle. Concentration-response curves to direct stimulation of cyclic AMP-dependent protein kinase using dibutyryl cyclic AMP were also shifted parallel to the right in left and right ventricular papillary muscles of isoprenaline-treated rats with 3.2 and 2.1 fold shifts respectively. There was also a 42% reduction of the maximal response to dibutyryl cyclic AMP in left papillary muscle. Hence, β-AR-mediated desensitization in the ventricles is associated with a significant impairment in signalling distal to adenylate cyclase. Previous studies demonstrated an inability of phosphodiesterase inhibitors to fully reverse β-AR desensitization in ventricular myocytes from guinea-pigs chronically infused with noradrenaline (Wynne *et al*., 1993). In addition there are impaired responses of ventricular myocytes to dibutyryl cyclic AMP in noradrenaline-treated guinea-pigs (Jones *et al*., 1990) pointing to alterations in signalling distal to adenylate cyclase. Reduced responsiveness of ventricular papillary muscles to the phosphodiesterase inhibitor IBMX may also indicate alterations in the rate of metabolism of cyclic AMP although reduced production of cyclic AMP would be expected to cause similar effects.

Most studies of β-AR desensitization in the heart have focussed on changes at the level of receptors and G-proteins. A common feature of prolonged exposure to catecholamines is an increase in expression and activity of inhibitory G-proteins. This was first shown in rat cardiac myocytes exposed to noradrenaline (Reithmann *et al*., 1989; 1990) and subsequently in rats infused with isoprenaline (100 μg kg^{-1} h^{-1} for 4 days) in which increased expression of Giα2 and Giα3 was observed without change to Gsα (Eschenhagen *et al*., 1991; Mende *et al*., 1992). It would be of interest to examine the effects of ADP-ribosylation of Giα with pertussis toxin on β-AR-mediated responses in atrial and ventricular tissues from rats infused with isoprenaline. It is unlikely, however, that inotropic responses to β_1-AR stimulation would be fully restored by pertussis toxin treatment since in the current study desensitization was detectable distal to adenylate cyclase.

Desensitization of the cardiac β-AR-cyclic AMP signalling pathway distal to adenylate cyclase has previously received little attention. The magnitude of the desensitization of responses to dibutyryl cyclic AMP indicate that at least following 14 days exposure to a relatively high infusion rate of isoprenaline (400 μg kg^{-1} h^{-1}), impairments in the signalling pathway distal to adenylate cyclase contribute significantly to β-AR desensitization. Whether the functional changes observed in the present study are a result of isoprenaline-induced desensitization *per se*, or are secondary to the isoprenaline-induced hypertrophy, has not been addressed here. Regardless of this, the current findings are relevant to reports of impaired contractile responses to dibutyryl cyclic AMP of myocytes isolated from human failing hearts (Harding *et al*., 1992), a condition in which the level of circulating noradrenaline is raised and in which hypertrophy is common (Thomas & Marks, 1978; del Monte *et al*., 1995; Ferguson, 1993).

In conclusion, the present study demonstrates differences in the pattern of desensitization in different regions of the heart following chronic infusion of isoprenaline. Right atrial chronotropic responses showed defects in signalling between the receptor and adenylate cyclase whereas inotropic responses of left atrium and ventricular papillary muscles exhibited an additional depression of responses to direct stimulation of cyclic AMP dependent protein kinase. None of the cardiac preparations showed altered contractility to Ca^{2+}. Although the molecular identity of proteins affected by desensitization has not been directly examined here, we are studying the expression of genes coding for a number of proteins in the cyclic AMP signalling pathway in atrial and ventricular tissues from isoprenaline-treated rats to provide further insights into the mechanisms involved.

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