Experimental Physiology – *Research Paper*

Voluntary exercise-induced changes in β_2-adrenoceptor signalling in rat ventricular myocytes

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Regular exercise is beneficial to cardiovascular health. We tested whether mild voluntary exercise training modifies key myocardial parameters [ventricular mass, intracellular calcium ($[Ca^{2+}]_i$) handling and the response to β-adrenoceptor (β-AR) stimulation] in a manner distinct from that reported for beneficial, intensive training and pathological hypertrophic stimuli. Female rats performed voluntary wheel-running exercise for 6–7 weeks. The mRNA expression of target proteins was measured in left ventricular tissue using real-time reverse transcriptase-polymerase chain reaction. Simultaneous measurement of cell shortening and $[Ca^{2+}]_i$ transients were made in single left ventricular myocytes and the inotropic response to β_1- and β_2-AR stimulation was measured. Voluntary exercise training resulted in cardiac hypertrophy, the heart weight to body weight ratio being significantly greater in trained compared with sedentary animals. However, voluntary exercise caused no significant alteration in the size or time course of myocyte shortening and $[Ca^{2+}]_i$ transients or in the mRNA levels of key proteins that regulate Ca^{2+} handling. The positive inotropic response to β_1-AR stimulation and the level of β_1-AR mRNA were unaltered by voluntary exercise but both mRNA levels and inotropic response to β_2-AR stimulation were significantly reduced in trained animals. The β_2-AR inotropic response was restored by exposure to pertussis toxin. We propose that in contrast to pathological stimuli and to beneficial, intense exercise training, modulation of Ca^{2+} handling is not a major adaptive mechanism in the response to mild voluntary exercise. In addition, and in a reversal of the situation seen in heart failure, voluntary exercise training maintains the β_1-AR response but reduces the β_2-AR response. Therefore, although voluntary exercise induces cardiac hypertrophy, there are distinct differences between its effects on key myocardial regulatory mechanisms and those of hypertrophic stimuli that eventually cause cardiac decompensation.

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Regular exercise induces a range of morphological and functional cardiovascular adaptations to meet increased physical demand. These adaptations are thought to be beneficial, and increased activity in sedentary populations is recommended by health professionals (Fletcher *et al.* 1996). Animal models of exercise are useful in the investigation of the cellular mechanisms associated with exercise-induced adaptations (Moore & Korzick, 1995). We wish to achieve a better understanding of the beneficial effects upon the heart of the regular, mild exercise that is recommended to the general population and how these changes may contrast with changes reported for pathological hypertrophic stimuli, such as hypertension, and the beneficial changes seen in response to intensive training seen in athletes.

One outcome of regular exercise is cardiac hypertrophy, and we have previously described concentric hypertrophy and increased force production in single myocytes in response to several weeks of voluntary wheel running in female rats (Natali *et al.* 2002). Initial compensated cardiac hypertrophy also occurs in response to pathological hypertrophic stimuli, such as hypertension, where an

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increase in foetal gene expression, the release of neurohormonal factors, including naturetic peptides, and an increase in inflammatory cytokines accompany the increase in myocardial mass. In addition, there is a modification of intracellular Ca^{2+} handling such that an increase in the amplitude of the intracellular Ca^{2+} transient and, as a consequence, an increase in myocyte shortening occur (Brooksby *et al.* 1993; Gómez *et al.* 1997). Changes in Ca^{2+} handling are also seen in response to exercise training (Wisloff *et al.* 2001; Kemi *et al.* 2007, 2008). Interestingly, these occur in a graded manner, dependent upon exercise intensity (Kemi *et al.* 2005).

In heart failure, enhanced sympathetic tone and thus sustained β-adrenoceptor (β-AR) activation initially provide the required contractile compensation for patients with ventricular impairment. However, over time the contractile response to continued β-AR stimulation becomes desensitized, resulting in a selective downregulation of β_1-AR density and a higher β_2:β_1 receptor ratio (Bristow *et al.* 1986; Xiao *et al.* 2003). Similar observations have been reported in cases of exercise-induced hypertrophy in rats (Barbier *et al.* 2004), although not all studies share these findings (Werle *et al.* 1990; Sylvestre-Gervais *et al.* 1982). However, these studies used enforced, intense exercise regimes that may cause stress adaptations in addition to exercise-induced adaptations (Yancey & Overton, 1993; Moraska *et al.* 2000; Brown *et al.* 2007). Importantly, with regard to β-AR signalling, increased levels of circulating catecholamines and increased adrenal weight are well-established responses to stress (Moraska *et al.* 2000).

In this study, we have used a voluntary wheel-running rat model to test the null hypothesis that (in contrast to pathological hypertrophic stimuli) changes in the expression of hypertrophic markers and Ca^{2+} handling do not occur in response to mild, voluntary exercise. Additionally, the effect of voluntary exercise training on the response to β-AR stimulation has not previously been tested; thus, the second null hypothesis was that the response to β-AR stimulation is unaltered by voluntary exercise training.

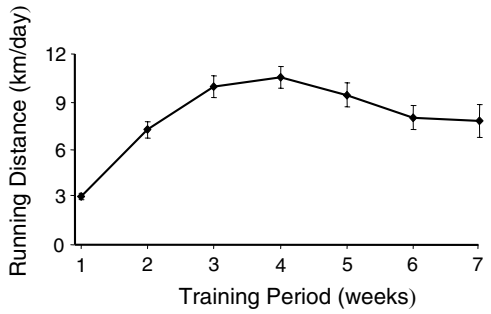

Figure 1. Average daily running distance in voluntarily exercising female rats
Running distance increased gradually over the first 4 weeks of training, from 3.03 ± 0.2 km day^{-1} in week 1 to 10.53 ± 0.69 km day^{-1} by week 4. Data from 31 animals.

Table 1. Heart weight to body weight (HW:BW), ventricular weight to body weight (VW:BW) and left ventricular weight to body weight ratios (LVW:BW) for trained and sedentary rats

	Sedentary	Trained
HW:BW (mg g^{-1})	5.04 ± 0.14 ($n = 30$)	5.66 ± 0.12* ($n = 31$)
VW:BW (mg g^{-1})	4.05 ± 0.15 ($n = 20$)	5.18 ± 0.09* ($n = 21$)
LVW:BW (mg g^{-1})	1.9 ± 0.07 ($n = 19$)	2.49 ± 0.08* ($n = 20$)

There is a statistically significant increase in HW:BW, VW:BW and LVW:BW ratios in trained compared with sedentary rats (*$P < 0.001$, Student's unpaired t test; n is the number of animals).

Methods

Ethical approval

The investigation conforms to the UK Animals (Scientific Procedures) Act 1986.

Exercise training model

Female Sprague–Dawley rats were weight- and age-matched and assigned to either a sedentary (SED) or trained group (TRN). Female rats have been shown to be more likely than males to run spontaneously (e.g. Rodnick *et al.* 1992). Rats were housed individually in plastic cages, and TRN rats had free access over a 6–7 week period to a vertical running wheels attached to their cages (Natali *et al.* 2001, 2002). Daily running distance was recorded. All animals were housed at 21°C with a 12 h–12 h light–dark cycle and had access to standard rat chow and water *ad libitum*.

Cardiac myocyte isolation and contractility

Following the training period, rats were weighed and killed humanely by cervical dislocation following stunning using Schedule 1 procedures sanctioned by the UK Home Office. The heart was rapidly removed and extraneous tissue dissected away. The heart was flushed with a modified Hepes–Tyrode solution of the following composition (mM): 130 NaCl, 5.4 KCl, 1.4 $MgCl_2$, 0.4 NaH_2PO_4, 0.75 $CaCl_2$, 5 Hepes, 10 glucose, 20 taurine and 10 creatine, pH 7.4 with NaOH at room temperature (20–22°C). The heart was then blotted and weighed before being mounted onto a Langendorff perfusion apparatus for isolation of myocytes using a collagenase–protease dispersion technique (Calaghan *et al.* 1998). At the end of the perfusion, the ventricles were separated and weighed. Single myocytes from the left ventricular free wall were isolated by mechanical

Table 2. Comparison of mRNA expression in the left ventricle of trained (TRN) and sedentary rats (SED) for putative hypertrophc triggers and pathological hypertrophy and inflammatory markers

Target	Sedentary	Trained	*P* value
Hypertrophic triggers			
Angiotensin II receptor 1A			
Agtr1a-Rn00578456_m1	1.09 ± 0.06	1.14 ± 0.08	n.s. 0.701
Endothelin-1			
Edn1-Rn00561129_m1	1.05 ± 0.09	0.89 ± 0.10	n.s. 0.245
Endothelin-1 receptor type A			
Ednra-Rn00561137_m1	0.97 ± 0.04	1.00 ± 0.03	n.s. 0.518
Endothelin-1 receptor type B			
Ednrb-Rn00569139_m1	1.18 ± 0.08	1.46 ± 0.08	TRN > SED 0.027
Insulin-like growth factor 1			
Igf1-Rn00710306_m1	1.25 ± 0.06	1.18 ± 0.09	n.s. 0.559
Insulin-like growth factor 1 receptor			
Igf1r-Rn00583837_m1	1.09 ± 0.03	1.16 ± 0.07	n.s. 0.381
Insulin-like growth factor 1 binding protein			
Igfbp3-Rn00561416_m1	1.33 ± 0.07	1.25 ± 0.08	n.s. 0.441
Pathological hypertrophy and inflammatory markers			
β-Myosin heavy chain			
Myh7-Rn00568328_m1	1.26 ± 0.07	1.11 ± 0.18	n.s. 0.554
Atrial natriuretic peptide			
Nppa-Rn00561661_m1	0.87 ± 0.20	1.19 ± 0.33	n.s. 0.405
Brain natriuretic peptide			
Nppb-Rn00580641_m1	1.55 ± 0.17	1.26 ± 0.15	n.s. 0.211
Tumour necrosis factor α			
Tnf-Rn00562055_m1	1.39 ± 0.09	0.90 ± 0.13	SED > TRN 0.007

Assay identification reference is given below target's common name. Data are from $n = 10$ hearts from trained and 10 from sedentary rats. *P* values are the probability derived from Student's unpaired *t* test; n.s. not significant.

dispersion. Cells were stored at 20–22°C in the modified Hepes–Tyrode solution and used within 8 h.

Following isolation, cells were added to an experimental chamber (volume 0.5 ml) on the stage of an inverted microscope (Nikon, Japan) and superfused at 37°C with solution of the following composition (mM): 135 Na^+, 5 K^+, 1 Mg^{2+}, 102 Cl^-, 20 HCO_3^{2-}, 1 SO_4^{2-}, 1 Ca^{2+}, 20 acetate, 10 glucose and 5 units l^{-1} insulin. This solution was equilibrated with 5% CO_2–95% O_2 to give a pH of 7.35. Quiescent, rod-shaped cells were field stimulated to contract at a rate of 1 Hz with a pulse width of 5 ms. Simultaneous measurement of myocyte contractility and intracellular Ca^{2+} ($[Ca^{2+}]_i$) transients was carried out as previously described (Natali *et al.* 2002). Briefly, myocyte cell shortening was measured, using a video-edge detection system and expressed as a percentage of resting cell length. Cells were loaded with the fluorescent Ca^{2+} indicator fura 2 (acetoxymethyl ester form, 3 μM for 10 min) and illuminated alternately with light of 340 and 380 nm. The resultant fluorescence was collected at 510 ± 20 nm and $[Ca^{2+}]_i$ indexed as the 340:380 ratio. The time course of contraction and $[Ca^{2+}]_i$ transient amplitudes was monitored as the time from stimulation to peak (in ms) and as the rate of decline in contraction (in $\mu m\ s^{-1}$) and $[Ca^{2+}]_i$ (in ratio units s^{-1}) from peak to half-peak levels.

When basal contractile activity was established, cell shortening in response to either β_1-AR stimulation [10^{-7} M ICI 118,551 in combination with 10^{-7} M isoprenaline (ISO)] or β_2-AR stimulation (5×10^{-5} M salbutamol in the presence of 10^{-7} M atenolol) or both (10^{-9}–10^{-6} M isoprenaline) was recorded (Xiao & Lakatta, 1993). The effect of these agents was expressed as change in a monitored parameter, relative to the predrug value. To inhibit the inhibitory α−subunits of GTP-activated protein ($G\alpha_i$), myocytes were incubated with pertussis toxin (PTX) for 2 h at 37°C at a concentration of 1.5 $\mu g\ ml^{-1}$ (Xiao *et al.* 1999).

Isolation of RNA

Left ventricular free wall tissue samples were excised from 10 trained and 10 sedentary female Sprague–Dawley rat hearts, snap frozen in liquid nitrogen between the large flat ends of a pair of tongs and samples harvested on a cryostat. All samples were stored at −80°C until RNA extraction. Total RNA extraction was performed using a modified Qiagen mini-kit protocol for striated muscle (Wittwer *et al.* 2002). The cryostat sections were homogenized (rotorstrator homogenizer, Ultra Turrax T8, IKA, Staufen, Germany) in 666 μl RLT–β-mercaptoethanol lysis buffer for 2 min. diethylpyrocarbonate (DEPC)-treated

water and proteinase K was added to give a final volume of 2 ml (final concentration 24 mAU $ml^{-1} \approx 0.8$ mg ml^{-1}; Qiagen, Hilden, Germany) and the mixture incubated at 45°C for 1.5–2 h. Samples were then centrifuged at 17949 g at 20–22°C for 5 min and the supernatant retained. Two millilitres of RLT–β-mercaptoethanol was added prior to 2 ml ethanol before binding of the RNA to the Qiagen Mini-Column. DNAse was added to the column to prevent contamination from co-extracted DNA (RNAse free kit, Qiagen). Samples of RNA were separated by electrophoresis through 0.6% formaldehyde–1% agarose gels and visualized by ethidium bromide staining, and the concentration of RNA was adjusted to 1 $\mu g\ \mu l^{-1}$. Cloned DNA was prepared from 4 μg of total RNA with random priming using Superscript III first-strand synthesis system (Invitrogen, Life Technologies, Rockville, MD, USA) and diluted 1:10 in Tris/EDTA buffer before use in real-time reverse transcriptase-polymerase chain reaction (RT-PCR).

Real-time RT-PCR

Real-time RT-PCR was performed using TaqMan low-density arrays (Micro Fluidic Cards, Applied Biosystems, Foster City, CA, USA) using the ABI RISM 7900HT sequence detection system (Applied Biosystems; Stones *et al.* 2007). Each card consists of 384 wells, preloaded with predesigned fluorogenic TaqMan probes and primers, configured to allow detection of selected transcripts for eight samples. The probes were labelled with a fluorescent reporter dye, 6-carboxyfluorescein (FAM, Applera Corp., Norwalk, CT, USA), on the 5′ end and with

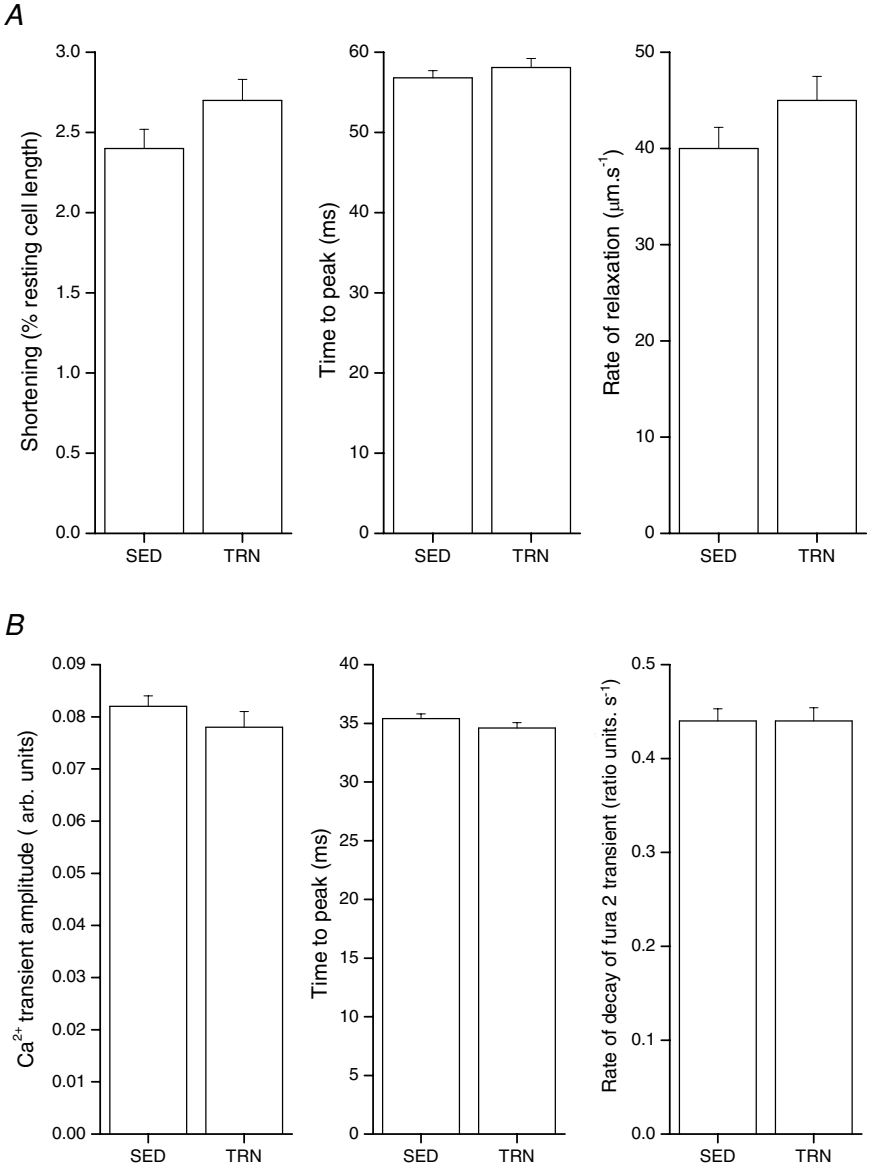

Figure 2. Cell shortening and intracellular Ca^{2+} transients in myocytes isolated from sedentary and trained rats
A, amplitude of shortening expressed as a percentage of resting cell length, time from stimulation to peak shortening and rate of relaxation. *B*, amplitude of Ca^{2+} transient, time from stimulation to peak transient and rate of transient decline. Parameters were not significantly different between trained (TRN) and sedentary (SED) rat myocytes ($P > 0.05$, Student's unpaired *t* test, data from 139 SED and 126 TRN myocytes).

Table 3. Comparison of mRNA expression in the left ventricle of trained (TRN) and sedentary rats (SED) for Ca^{2+} handling proteins and adrenoceptors

Target	Sedentary	Trained	*P* value
Calcium handling			
Sarco-Endoplasmic Reticulum Ca^{2+}-ATPase type 2A			
Atp2a2-Rn00568762_m1	1.01 ± 0.02	1.06 ± 0.05	n.s. 0.365
L-type Ca^{2+} channel 1C			
Cacna1c-Rn00709287_m1	1.34 ± 0.04	1.40 ± 0.05	n.s. 0.295
Phospholamban	1.23 ± 0.07	1.23 ± 0.07	n.s. 0.942
Ryanodine receptor 2			
Ryr2-Rn01470303_m1	1.19 ± 0.08	1.21 ± 0.07	n.s. 0.819
Ca^{2+}–calmodulin-dependent protein kinase II			
Camk2d-Rn00560913_m1	0.93 ± 0.01	0.93 ± 0.02	n.s. 0.869
Calsequestrin 2			
Casq2-Rn00567508_m1	1.04 ± 0.02	1.06 ± 0.03	n.s. 0.566
Sodium–calcium exchanger			
Slc8a1-Rn00570527_m1	1.05 ± 0.06	1.09 ± 0.09	n.s. 0.746
Adrenoceptors			
α_{1a}-Adrenergic receptor			
Adra1a-Rn00567876_m1	1.00 ± 0.12	0.95 ± 0.07	n.s. 0.69
β_1-Adrenergic receptor			
Adrb1-Rn00824536_s1	1.27 ± 0.06	1.19 ± 0.08	n.s. 0.427
β_2-Adrenergic receptor			
Adrb2-Rn00560650_s1	1.21 ± 0.05	1.06 ± 0.04	SED > TRN 0.034

Assay identification reference is given below target's common name. Data are from $n = 10$ hearts from trained and 10 from sedentary rats. *P* value is the probability derived from Student's unpaired *t* test; n.s. not significant.

non-fluorescent quencher on the 3′ end. cDNA containing the equivalent of 77 ng of RNA was mixed with 50 μl of TaqMan Universial PCR master mix (Applied Biosystems) to give a final volume of 100 μl and loaded into each well. The Microfluidic cards were thermal cycled at 50°C for 2 min and 94.5°C for 10 min, followed by 40 cycles at 97°C for 30 s and 59.7°C for 1 min. Data were collected with instrument spectral compensations by the Applied Biosystems SDS 2.2 software, and analysed using the threshold cycle (C_T) relative quantification method, data being normalized to an aggregate of 36 transcripts rather than GAPDH or 18S alone to reduce data variability and increase reproducibility (Livak & Schmittgen, 2001).

Statistics

All data are expressed as means ± S.E.M. Statistical analysis was performed by using Student's paired or unpaired *t* test as appropriate or equivalent non-parametric tests if data were not normally distributed. Statistical significance was assumed with a probability of less than 0.05.

Results

Average daily running distance increased gradually over the first 4 weeks of training from 3.0 ± 0.2 km day^{-1} in week 1 to 10.5 ± 0.7 km day^{-1} by week 4 (Fig. 1) and resulted in cardiac hypertrophy, as confirmed by a significant increase in heart weight to body weight ratio, ventricular weight to body weight ratio and left ventricular weight to body weight ratio (Table 1).

Effect of voluntary exercise on hypertrophic gene expression and Ca^{2+} handling

The insulin-like growth factor 1 (IGF-1) signalling pathway is implicated in the hypertrophic response of skeletal muscle in response to exercise, while the endothelin-1 (ET-1) signalling pathway is associated with cardiac hypertrophy in response to hypertension. The mRNA expression for the IGF-1 signalling pathway was not increased by voluntary exercise. There was a significant increase in mRNA expression for the B-type ET-1 receptor in TRN hearts but not in mRNA expression for other proteins associated with ET-1 signalling (Table 2). Markers of hypertrophy associated with pathological sequalae, for example atrial and brain natriuretic peptides and β-myosin heavy chain, were unaltered by exercise, while the mRNA for the inflammatory cytokine tumour necrosis factor α (TNF-α) was significantly decreased in TRN (Table 2).

Unloaded cell shortening and $[Ca^{2+}]_i$ were simultaneously recorded in left ventricular myocytes. There were no significant differences in the amplitude or time course of cell shortening or $[Ca^{2+}]_i$ transient between SED ($n = 139$) and TRN myocytes ($n = 126$; Fig. 2). Consistent with these observations, there were

no differences in the mRNA expression of myocyte Ca^{2+} handling targets likely to influence these parameters (Table 3).

Effect of voluntary exercise on the response to β-AR stimulation

Exposure to ISO (which stimulates both β_1- and β_2-ARs) caused a concentration-dependant positive inotropic (increase in cell shortening and $[Ca^{2+}]_i$ transient amplitude) and lusitropic effects (increase in rate of relaxation and decline of $[Ca^{2+}]_i$ transient) in all cells (Fig. 3*A* and *B*). The effects upon the time course of contraction and $[Ca^{2+}]_i$ transient were not significantly different between SED and TRN myocytes. However, the concentration–response curves for both contraction and $[Ca^{2+}]_i$ transients differed between SED and TRN myocytes (Fig. 3*C* and *D*; data from 41 SED and 41 TRN myocytes). There appeared to be no difference in the sensitivity of myocytes to ISO, since the half-maximal concentrations (EC_{50}) were not significantly different (contraction, SED 12.5 ± 1.41 nM and TRN

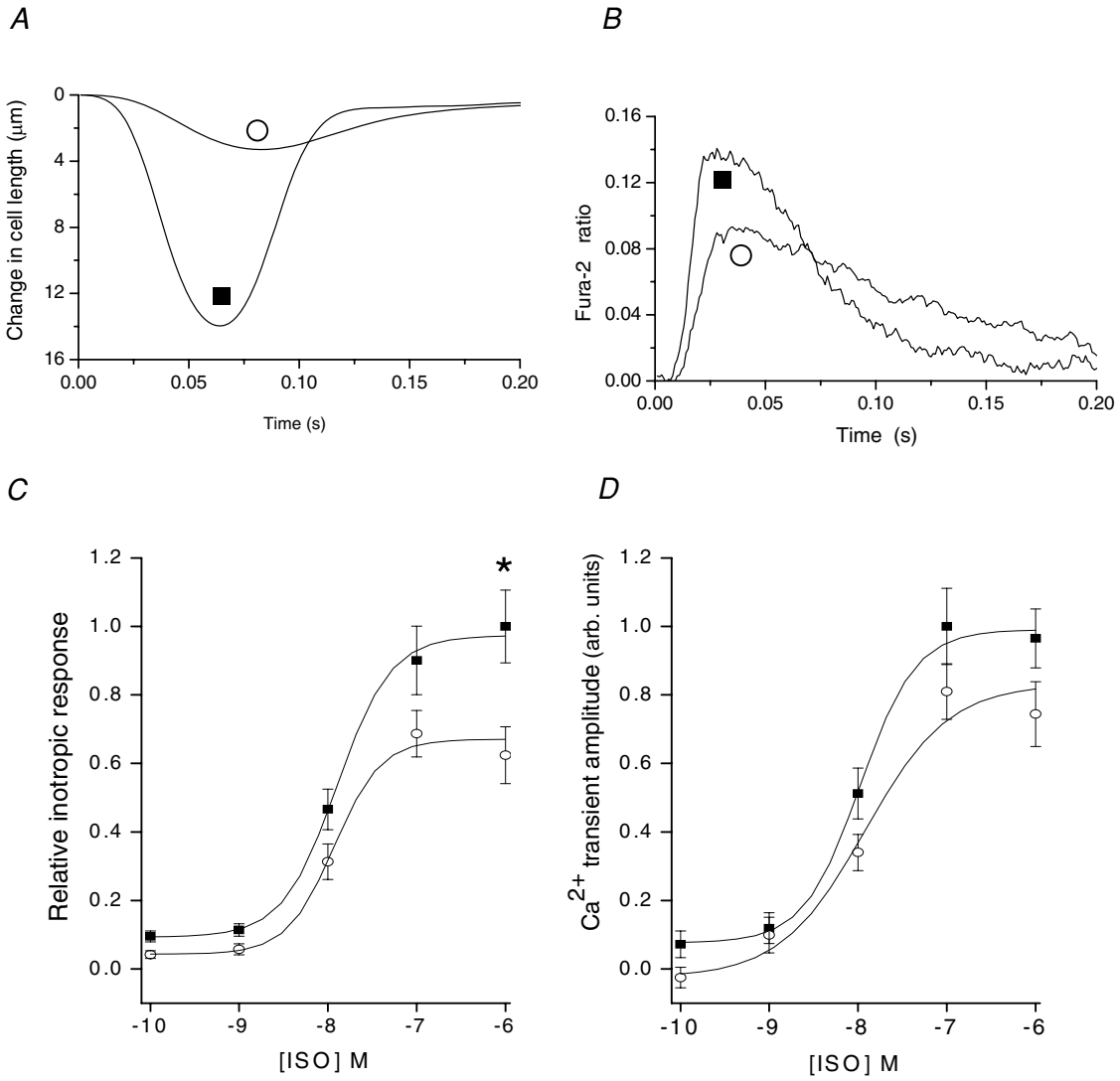

Figure 3. Effect of isoprenaline on contraction and on the Ca^{2+} transient of rat left ventricular myocytes
A, contraction expressed as change in cell length in the absence (○) and after 1 min exposure to 10^{-7} M isoprenaline (■). *B*, fura−2 fluorescence ratio in the same cell as in *A* in the absence (○) and after 1 min exposure to 10^{-7} M isoprenaline (■). *C* and *D*, dose–response curves for the effect of isoprenaline on contraction (*C*) and on the Ca^{2+} transient (*D*) of myocytes from sedentary (■) and trained rats (○). Values are normalized to maximal effect in SED myocytes. There was no significant difference in the EC_{50} values (concentration giving half maximal effect) between SED and TRN myocytes but the maximal response for shortening was significantly reduced in TRN myocytes (see text for values; $^*P = 0.007$, Student's unpaired *t* test, data from 41 SED and 41 TRN myocytes).

11.7 ± 2.44 nM; $[Ca^{2+}]_i$, SED 10.5 ± 1.68 nM and TRN 11.9 ± 8.25 nM; $P > 0.05$). The maximal increase in the amplitude of contraction was significantly depressed in TRN myocytes to 0.62 ± 0.08 of the maximal response seen in SED myocytes ($P = 0.007$). The maximal increase in $[Ca^{2+}]_i$ was smaller in TRN compared with SED (0.81 ± 0.08, $P > 0.05$) but this did not reach statistical significance.

We observed that levels of mRNA expression of the β_1-AR were unaltered but levels of β_2-AR were significantly lower in TRN myocardium (Table 3). Measurement of β_1- and β_2-AR protein levels in TRN and SED samples by Western blotting were not conclusive. We therefore tested whether the different response to mixed $\beta-$AR stimulation might result from differential effects upon β_1- and β_2-ARs. As expected, the inotropic and lusitropic response to β_1-AR stimulation was greater than that to β_2-AR stimulation (Fig. 4*A* and *B*). The lusitropic effects of β-AR stimulation were not altered by exercise (rate of relaxation in SED $n = 39$ myocytes, 92 ± 20 to $248 \pm 30\ \mu m\ s^{-1}$ and TRN $n = 54$ myocytes, 110 ± 10 to $270 \pm 20\ \mu m\ s^{-1}$ following β_1-AR stimulation; SED $n = 54$ myocytes, 89 ± 10 to $148 \pm 20\ \mu m\ s^{-1}$ and TRN $n = 47$ myocytes, 99 ± 10 to $128 \pm 20\ \mu m\ s^{-1}$ following β_2-AR stimulation; Fig. 4*C*). The positive inotropic response to selective β_1-AR stimulation was unaltered by exercise (Fig. 5*A*). In contrast, cell shortening in response to selective β_2-AR stimulation was significantly reduced in myocytes isolated from TRN animals compared with SED animals (Fig. 5*B*).

β_2-Adrenoceptor stimulation causes activation of both stimulatory ($G\alpha_s$) and inhibitory ($G\alpha_i$) α-subunits of GTP-activated protein. We tested whether the reduced inotropic response to β_2-AR might be caused by increased activity of $G\alpha$ by prior treatment with the $G\alpha_i$ inhibitor, pertussis toxin (PTX). Consistent with this hypothesis, the depressed contractile response to β_2-AR stimulation was relieved by PTX treatment (Fig. 5*B*).

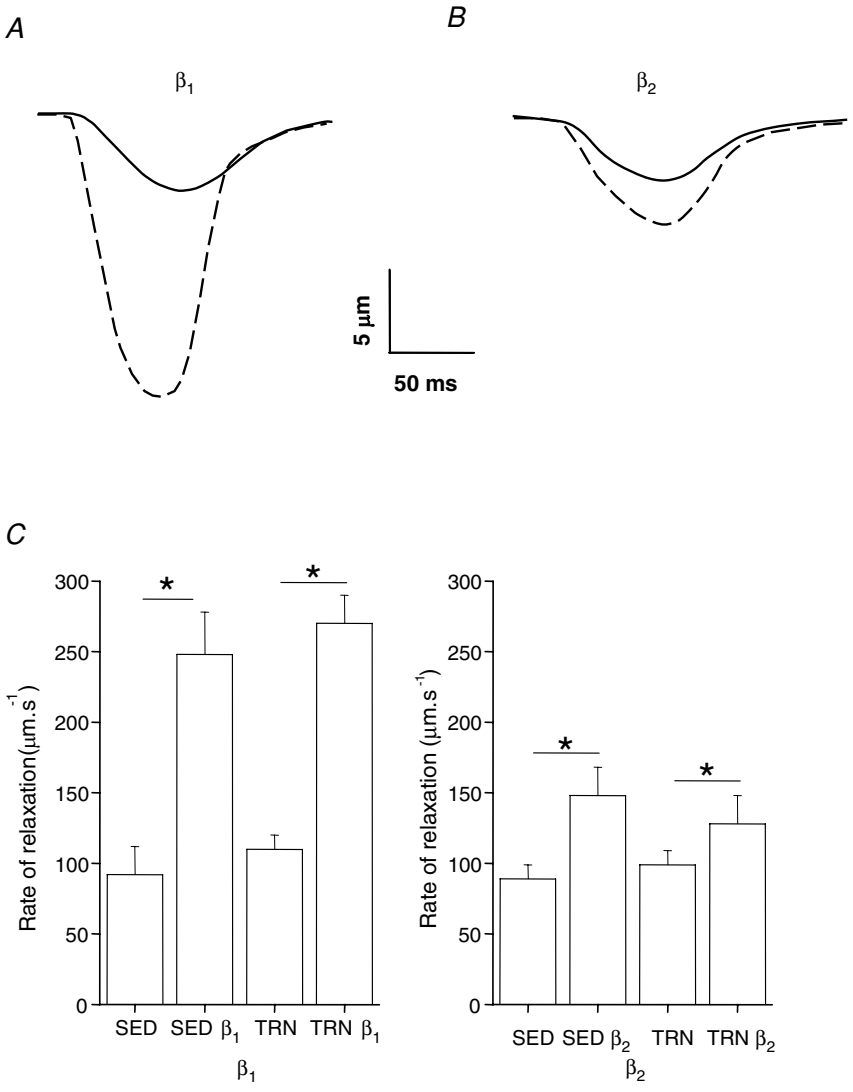

Figure 4. Unloaded cell shortening in response to β_1- and β_2-AR stimulation
A and *B*, cell shortening recorded from representative myocytes isolated from a sedentary rat heart. *A*, shortening under control conditions (continuous line) and in response to β_1-AR stimulation (10^{-7} M isoprenaline in the presence of 10^{-7} M ICI 118,551, dashed line). *B*, shortening under control conditions (continuous line) and in response to β_2-AR stimulation (5×10^{-5} M salbutamol in the presence of 10^{-7} M atenolol, dashed line). The largest positive inotropic effect is seen in response to β_1-AR stimulation. *C*, rate of relaxation was significantly greater in response to β_1- than β_2-AR stimulation in both trained and sedentary rat myocytes (*$P < 0.001$, Student's paired *t* test).

Discussion

Consistent with our previous findings using the voluntary wheel-running rat model (Natali *et al.* 2001, 2002), cardiac hypertrophy occurs in response to voluntary exercise. The type of exercise undertaken involves multiple short periods of running interspersed with short breaks under a low load associated with the resistance and inertia of the wheel. This results in concentric hypertrophy of the myocytes. Recent analysis (Naylor *et al.* 2008) indicates that in humans concentric hypertrophy can arise in response to both resistance and endurance training.

Endothelin 1 is implicated in the development of pathological hypertrophy resulting from haemodynamic overload (Arai *et al.* 1995; Yamazaki *et al.* 1996; Serneri *et al.* 1999). Whilst there was no change in the molecular correlates of ET-1 receptor type A in our model of physiological cardiac hypertrophy, there was a significant increase in the ET-1 B receptor. The latter constitute only 25% of the total population of endothelin receptors in the rat left ventricle (Thibault *et al.* 1995).

In the heart, the insulin-like growth factor (IGF) and phosphoinositide 3-kinase (PI3-kinase) pathway has been linked with normal cardiac development and the generation of physiological hypertrophy in response to treadmill training (McMullen *et al.* 2003, 2004). When the IGF receptor 1 is overexpressed, compensated hypertrophy ensues that is similar to physiological hypertrophy (McMullen *et al.* 2004). However, the gene expression of the IGF-1 signalling pathway was not increased in our model of exercise-induced hypertrophy. Our experimental design imposed a single monitoring point for mRNA levels at 6–7 weeks; therefore, it is possible that an earlier sampling point when daily running is increasing rapidly may reveal changes in the expression of the genes responsible for the hypertrophic response.

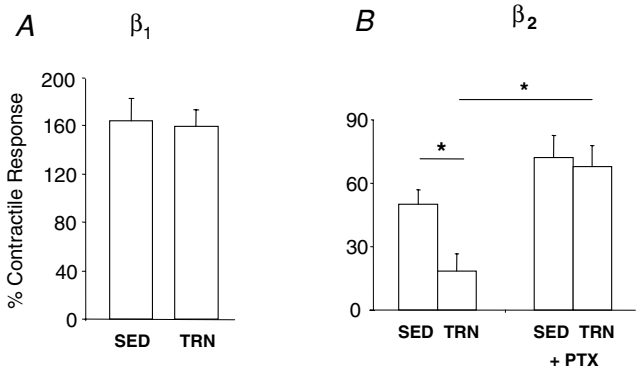

Figure 5. The effect of exercise training on the response to β-AR stimulation
Mean data for cell shortening expressed as a percentage of resting cell length in left ventricular myocytes isolated from trained and sedentary rats. *A*, the positive inotropic response to β_1-AR stimulation was unaltered by exercise ($n = 39$ SED, $n = 54$ TRN, $P > 0.05$, Student's unpaired *t* test). *B*, the positive inotropic response to β_2-AR stimulation was significantly reduced in left ventricular myocytes isolated from trained rats compared with sedentary rats ($n = 54$ SED, $n = 47$ TRN, $P = 0.003$, Student's unpaired *t* test). Following incubation with PTX, the inotropic response to β_2-AR stimulation was enhanced in left ventricular myocytes isolated from sedentary and trained rats and there was no significant difference in the contractile response to β_2-AR stimulation between the two groups ($n = 32$ SED, $n = 31$ TRN, $P > 0.05$, Student's unpaired *t* test).

Upregulation of the gene expression of atrial and brain natriuretic peptides and β-myosin heavy chain are hallmark features of pathological hypertrophy (Kinnunen *et al.* 1993; Bhatia *et al.* 2003). The expression of these genes was unchanged in TRN hearts, demonstrating either distinct signalling pathways for physiological and pathological hypertrophy or, alternatively, that the severity of the physiological hypertrophic signals is less than that induced during pathological conditions. Furthermore, the inflammatory cytokine, TNF-α, was significantly reduced in TRN compared with SED. Cytokines are indicative of the inflammatory status of a tissue (Kapadia *et al.* 1995; Sheng *et al.* 1997; Gwechenberger *et al.* 1999). Tumour necrosis factor α can stimulate the hypertrophy and apoptosis of cultured cardiac myocytes, while transgenic mice that overexpress a secreted form of TNF-α develop concentric hypertrophy that progresses to dilated cardiomyopathy (Kubota *et al.* 1997; Sivasubramanian *et al.* 2001). Thus, voluntary moderate exercise improved the inflammatory status of the myocardium.

Previous studies have reported changes in Ca^{2+} handling in hypertension-induced cardiac hypertrophy that result in increased contraction, e.g in spontaneously hypertensive rats (Brooksby *et al.* 1993; Gómez *et al.* 1997; McCrossan *et al.* 2004). Enforced, intense exercise training protocols have also been reported to alter myocyte Ca^{2+} transients and contraction (Wisloff *et al.* 2001; Kemi *et al.* 2007, 2008). These changes are graded, with smaller or no changes seen in some parameters with moderate enforced exercise regimes (Kemi *et al.* 2005). Our findings are consistent with this grading of response, since we find that mild exercise causes no change in Ca^{2+} handing or cell shortening or in the gene expression of the major Ca^{2+} handling proteins. These findings also support our earlier conclusion that increased absolute force development from such myocytes results principally from the increase in their cross-sectional area (Natali *et al.* 2002) rather than increased levels of $[Ca^{2+}]_i$. Thus, we predict that while all levels of exercise will result in increased myocardial mass, intense training undertaken by human athletes will additionally modify Ca^{2+} handling whilst mild exercise in the general population will not.

In normal myocardium, there is a greater density of β_1-AR than β_2-AR in both humans (77%:23%; Bristow *et al.* 1986) and rats (56%:44%; Xiao *et al.* 2003). Several studies have reported a decrease in ventricular β-AR density and the contractile response of myocytes to β-AR stimulation in rats subjected to a variety of training regimes

(Sylvestre-Gervais *et al.* 1982; Takeda *et al.* 1985; Plourde *et al.* 1991; Nieto *et al.* 1996). Werle *et al.* (1990) reported a swim-training induced decrease in β_1- and β_2-ARs with no affect on the ratio of β_1 to β_2. More recently, Barbier *et al.* (2004) reported a training-induced decrease in β_1-AR with no modification of β_2-AR following an 8 week treadmill training protocol in rats. In contrast, other studies found no change in β-AR number in rats undertaking treadmill running regimes (Moore *et al.* 1982; Bohm *et al.* 1993; Scarpace *et al.* 1994; Roth *et al.* 1998) or swim training (Williams, 1980; Williams *et al.* 1984). Subtle differences in methodological approaches and the potential effect on circulating catecholamines that stressful, enforced exercise regimes can have (Yancey *et al.* 1993; Moraska *et al.* 2000, Brown *et al.* 2007) may explain the differences between observations in these studies.

In response to voluntary wheel-running exercise, we observed a training-induced decrease in response to mixed β-AR stimulation in left ventricular myocytes without an effect upon the EC_{50} of the contractile and $[Ca^{2+}]_i$ transient responses to isoprenaline. Investigation of β-AR subtypes revealed that this probably results from an effect upon β_2-AR rather than β_1-AR, given that we saw a decrease in β_2-AR mRNA and decreased contractile function in response to β_2-AR stimulation with unchanged β_1-AR responses. Although the change in β_2-AR mRNA might suggest a decrease in the number of β_2-ARs, the modulation of the inotropic response to β_2-AR stimulation was reversed by inhibition of $G\alpha_i$, suggesting that both the number of functional β_2-AR and postreceptor signalling are involved.

Interestingly, our observations are the reverse of the situation seen in chronic heart failure and pathological cases of cardiac hypertrophy, where continued exposure of the myocardium to excessive levels of circulating catecholamines diminishes β-AR function as a result of a decrease in the ratio of β_1- to β_2-AR (Bristow *et al.* 1986; Xiao *et al.* 2003).

When measuring mRNA levels, it must be acknowledged that this may not always indicate protein levels, because RT-PCR methods, used to quantify mRNA, measure total mRNA rather than the active, ribose-associated mRNA (Serikawa *et al.* 2003; MacKay *et al.* 2004). However, total protein levels are not always indicative of functional protein levels because of post-transcriptional variations in protein degradation or trafficking to active sites (Kornitzer & Ciechanover, 2000; Kostova & Wolf, 2003). Therefore, in addition to mRNA levels of selected targets, we measured the functional correlates (Ca^{2+} transients, inotropic response to β-AR stimulation) of relevant channel/receptor activity.

In summary, although mild voluntary exercise results in cardiac hypertrophy, there are distinguishing features between the response to voluntary exercise and to both pathological causes of hypertrophy and beneficial hypertrophy caused by intense exercise regimes. These include an absence in the modification of $[Ca^{2+}]_i$ handling and a targeting of β_2-AR rather than β_1-AR signalling.

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