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Intravenous IGF-I receptor antisense reduces IGF-IR expression and diminishes pressor responses to angiotensin II in conscious normotensive rats

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1 Given the variety of cardiovascular effects of insulin-like growth factor-I (IGF-I), we investigated the effects of a functional deficit in IGF-I signalling in the conscious rat cardiovascular system using intravenous IGF-I receptor antisense (AS, 0.5 nmol) treatment.

2 Insulin-like growth factor-I receptor (IGF-IR) immunoreactivity was reduced in IGF-IR AS-treated tail arteries. Western immunoblot analysis demonstrated a decrease in cardiac IGF-IR in IGF-IR AS-treated rats; treatment reduced the expression of IGF-IR to $83 \pm 6\%$ of that in samples from vehicle-treated rats, compared to $99 \pm 3\%$ for a control, full-mismatch oligonucleotide (MM-18) or 100% (vehicle).

3 IGF-IR AS treatment had no effect on resting blood pressure during the 14-day treatment period.

4 Pressor responses (as measured by increase in systolic arterial pressure) to angiotensin II (AngII) gradually decreased over 2 weeks treatment with IGF-IR AS (5×0.5 nmol per intravenous injection, 2 weeks), and were significantly reduced at treatment day 14 compared to day 7 (2.7-fold rightward shift). IGF-IR AS treatment caused a significant rightward shift in the angiotensin II (AngII) dose–response compared to both vehicle and full-mismatch treated rats (4.0-fold shift compared to vehicle, $P<0.01$, $n=6$–14).

5 There was a significant decrease in cardiac angiotensin II type 1 receptor (AT_1R) expression in AS-treated rats compared to vehicle-treated rats; cardiac AT_1R was decreased to $80 \pm 6\%$ in comparison to 100%. AT_1R immunoreactivity was also reduced in IGF-IR AS-treated tail arteries.

6 IGF-IR AS treatment resulted in structural changes in both the heart and aortae, with small but significant differences observed between left ventricle/bodyweight ratios of AS and both vehicle- and MM-18-treated rats ($n=8$, $P<0.05$). Aortic cross-sectional areas of AS-treated rats were significantly lower than MM-18- and vehicle-treated rats ($27.4 \pm 5.7\%$ reduction of vehicle-treated samples, $n=8$, $P<0.01$).

7 The results of this study suggest that an induced loss of IGF-IR, while not affecting resting blood pressure, has a predominantly inhibitory effect on vascular response to vasoconstrictor agents including angiotensin II. This may occur through downstream effects on AT1R expression, *via* modulation of the expression of receptors for other vasoactive signalling molecules, or *via* changes in myocyte proliferation.
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Abbreviations: AngII, angiotensin II; AS, antisense; AT_1R, angiotensin II type 1 receptor; CVS, cardiovascular system; ECL, enhanced chemiluminescence; IGF-I, insulin-like growth factor-I; IGF-IR, insulin-like growth factor-I receptor; NA, noradrenaline; VSMC, vascular smooth muscle cells

Introduction

Although most circulating insulin-like growth factor I (IGF-I) is produced by the liver, IGF-I is also produced locally in most cell types. IGF-I has acute effects on haemodynamic function, and longer-term trophic effects on cardiac and vascular smooth muscle. Both IGF-I and the IGF-I receptor (IGF-IR) are highly expressed in vascular smooth muscle cells (VSMC) (Bornfeldt *et al*., 1990; Delafontaine *et al*., 1991; Giannella-Neto *et al*., 1992; Delafontaine, 1995). IGF-I is a vasodilator both in experimental animals and humans. *In vivo*, acute or chronic systemic administration of IGF-I reduces total systemic vascular resistance and blood pressure in rats (Cittadini *et al*., 1996; Walsh *et al*., 1996). *In vitro*, IGF-I decreases rat aorta or tail artery contractile responses to KCl and noradrenaline (NA) (Walsh *et al*., 1996; Vecchione *et al*., 2001), and decreases responses to angiotensin II (AngII) in cultured rat mesangial cells (Inishi *et al*., 1994). In healthy human subjects, infusion of IGF-I has been found to increase forearm blood flow (Fryburg, 1996).

IGF-I is also involved in the regulation of expression of VSMC AngII type I receptor (AT_1R). Incubation of cultured VSMC with IGF-I has been shown to increase AT_1R expression (Muller *et al*., 2000); however, in diabetic wild-type mice, overexpression of IGF-I was found to decrease AngII

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and AT_1R, (Kajstura *et al*., 2001), and thus the effects of IGF-I on AT_1R expression appear to be dependent on the system state. IGF-I regulates expression of receptors for other vasoactive signalling molecules such as NA; upregulation of α_1-receptors was found in the rat aortic VSMC with IGF-I treatment (Hu *et al*., 1996).

A number of studies have examined the involvement of IGF-I in smooth muscle and cardiac hypertrophy; exogenous administration of IGF-I induces an hypertrophic response in vascular smooth muscle (Chen *et al*., 1994; Kamide *et al*., 2000), cardiac muscle (Pauliks *et al*., 1999) and increases myocardial protein and DNA synthesis in cultured cardiomyocytes (Fuller *et al*., 1992; Ito *et al*., 1993; Decker *et al*., 1995). The growth-promoting effects of IGF-I have been demonstrated to occur *via* IGF-IR (Gustafsson *et al*., 1999). In addition, IGF-I treatment has been shown to enhance the contractility of cultured neonatal cardiac myocytes and isolated cardiac muscle (Kinugawa *et al*., 1999).

We used IGF-IR antisense (AS) to determine whether interference with IGF-I signalling would have one or more of the following effects: (a) alter resting blood pressure, (b) alter pressor responses to AngII and NA *via* interactions described above or (c) produce changes in vascular or cardiac muscular thickness.

Methods

Experimental animals

The experiments were carried out using adult female Hooded Wistar rats weighing between 200 and 280 g at the start of the experiment. The animals were housed in North Kent Plastics cages with sawdust bedding, and maintained on a constant 12 h light–12 h dark cycle, at 18–22°C. Animals were given normal tap water and food in the form of Clark King ARM cubes *ad libitum*. Experiments were carried out in accordance with the Australian National Health and Medical Research Council code of practice, 1997.

Surgical procedure

Rats were anaesthetised with amylobarbital sodium (0.1 g kg^{-1}, i.p.). The left jugular vein and left carotid artery were cannulated with polyethylene (PE 50) tubing. The jugular vein was rinsed with physiological saline, and the carotid artery was rinsed with heparinised saline solution (100 UI ml^{-1}). The cannulae were tunnelled subcutaneously to the back of the neck where they exited the animal.

In vivo *effects of AS treatments*

Pressor responses to NA (10 ng kg^{-1}–30 μg kg^{-1}) and AngII (1.0 ng kg^{-1}–30 μg kg^{-1}) were recorded at 7, 9, 11 and 14 days postoperation. Pilot experiments showed no effects of AS treatment on days 1, 3 and 5, and also no blood pressure or heart rate response to IGF-I (1.0–500 μg kg^{-1}). Rats were randomly assigned to one of four treatment groups to receive treatment every second day for 14 days; three groups received AS oligonucleotide or mismatch (0.5 nmol bolus injection, i.v., *via* cannulated jugular vein), while the last group received an equal volume of vehicle. Group one received an AS oligonucleotide targeting the IGF-IR (AS, 5′-UCC-CAC-AGC-TGC-UGC-AAG-3′, with a modification of 1–6 2′OMe RNA, 7–12 Thioate DNA, 13–18 2′OMe RNA), targeting the coding region of the IGF type I receptor mRNA, the same region as an IGF-IR AS we used previously to specifically reduce IGF-IR in psoriatic epidermis (Wraight *et al*., 2000). Group two received a complete mismatch of IGF-IR AS (MM-18, 5′-CAC-ACU-CAG-CTG-GCG-CCA-3′), with the same modification as AS. Group three received a single-base mismatch of IGF-IR AS (MM-1, 5′-UCC-CAC-AG**T**-TGC-UGC-AAG-3′, with the same modification as the AS. Blood pressure from the carotid artery was recorded using a Gould Statham Physiological pressure transducer connected to a Power Lab System.

Western blotting

Frozen rat aortae and hearts were washed in phosphate-buffered saline (PBS) and crushed to powder using a mortar and pestle by adding liquid nitrogen. The powder was transferred to ice-cold buffer (50 mM HEPES, 150 mM NaCl, 1.5 mM $MgCl_2$, 10% glycerol, 1% triton X-100 and 20 mM PMSF) (5 ml buffer per 1 g wet tissue) for 30 min on ice and homogenised in a Polytron homogeniser (1 cm diameter probe) (at 4°C). The homogenate was centrifuged at 16 000 r.p.m. for 15 min at 4°C, and the supernatant was collected and stored at −70°C prior to use. The protein concentration of the tissue extract was determined by the Bradford Method (Bradford, 1976). An equal amount of total protein (100 μg) was loaded into each lane and run on 7% sodium dodecyl sulphate–polyacrylamide gel electrophoresis. Separated proteins were transferred to Immobilon P membrane (100 V, 1.5 h). The membrane was blocked for 1 h, at room temperature, with 5% skim milk powder in PBS plus 1% Tween20 (PBST). The membrane was then washed briefly with PBST and probed with an IGF-IRβ (C-20) rabbit polyclonal IgG (1 : 500) or AT_1R (N-10) rabbit polyclonal IgG (1 : 500) for 2 h prior to washing in PBST (3 × 5 min). The membrane was then exposed to anti-rabbit horseradish peroxidase-conjugated secondary antibodies (1 : 1500) for an additional 1 h, washed as above plus 1 × 5 min with PBS. Bands were detected by enhanced chemiluminescence (ECL).

Immunohistochemical studies

Tail arteries and aortae were fixed in a solution containing 4% paraformaldehyde in PBS for 2 h at 4°C, then washed in PBS containing 7% sucrose and 0.01% sodium azide (4 × 10 min) and stored in this solution for 48 h at 4°C. Tissues were embedded in Tissue-Tek (Vector Laboratories, Sydney, Australia), and were then snap frozen in liquid nitrogen and stored at −70°C. Cryostat cut sections (Leica CM1850 cryostat at −20°C, 10 μm) were thawed onto gelatine-coated slides. The sections were then incubated for 18–20 h at 4°C with an IGF-IRβ (C-20) rabbit polyclonal IgG (1 : 25) or AT_1R (N-10) rabbit polyclonal IgG (1 : 25). For negative control studies, primary antibody was replaced with an antibody diluting medium. After rinsing in PBS (4 × 10 min), tissue sections were incubated in biotinylated anti-rabbit immunoglobulin (1 : 250) for 30 min at room temperature. The sections were rinsed again and then incubated in Texas red streptavidin (1 : 250) for an additional 30 min, at room temperature. Tissues were then washed as above and mounted in 'Vectashield'. Sections were

examined with a fluorescence microscope. A SPOT RT slider digital camera and SPOT RT software (v. 3.5) run on a Compaq 9500 personal computer were used to capture and analyse photomicrographs. Aortic medial cross-sectional area was determined using the SPOT RT 'define area' function for three sections per animal, for each animal treated with AS, mismatch or vehicle. The observer was blinded as to the treatment group for all observations.

Data analysis and statistics

EC_{50} and confidence intervals were calculated using a computer program, GraphPad Prism 3.02 (GraphPad Software, San Diego, CA, U.S.A.). The effects of IGF-IR AS on pressor responses to AngII and NA were determined using one-way ANOVA, followed by Bonferroni's test for multiple comparisons. A P-value of <0.05 was considered to indicate statistical significance. Data in graphs are presented as mean $\pm$ standard error of the mean (s.e.m.) and n indicates the number of animals or tissues being studied.

There is a large range of n values obtained between groups. This was due to the difficulty in maintaining functional cannulated arteries over the full course of the experiment; and also because we obtained data from a number of vehicle-treated rats to validate the protocol, and then repeated the same experiment at the same time as the AS and mismatch treated rats. At least six data points were obtained per treatment group.

Materials

[Val5]-Angiotensin II, NA and horseradish peroxidase-conjugated antibody were obtained from Sigma-Aldrich, Inc. (Sydney, Australia). IGF-IR chimeric AS oligonucleotide (5′-UCC-CAC-AGC-TGC-UGC-AAG-3′, with a modification of 1-6 2′OMe RNA, 7-12 Thioate DNA, 13-18 2′OMe RNA) and IGF-IR AS oligonucleotide with single-base mismatch (5′-UCC-CAC-AG**T**-TGC-UGC-AAG-3′) were from Eurogentec (San Diego, U.S.A.); IGF-IR AS oligonucleotide with complete mismatch (5′-CAC-ACU-CAG-CTG-GCG-CCA-3′) was from Eurogentec S.A. (Seraing, Belgium). Immobilon P membrane was purchased from Millipore Corporation (Bedford, MA, U.S.A.). IGF-IRβ (C-20) rabbit polyclonal IgG and AT_1R (N-10) rabbit polyclonal IgG were from Santa Cruz Biotechnology (CA, U.S.A.). The Enhanced Chemiluminescence Western blotting detector reagents were from Amersham Bioscience Pty Ltd (Baulkham Hills, Australia). Biotinylated anti-rabbit immunoglobulin, Texas red, streptavidin and Vectashield were purchased from Vector Laboratories (Sydney, Australia).

Results

Effect of IGF-IR AS on IGF-IR expression in rat tail artery

The IGF-IR expression levels in the rat tail arteries of IGF-IR AS and control treatments were examined using immunohistochemistry. Figure 1a–c shows the expression of IGF-IR in IGF-IR AS-, MM-18- and vehicle-treated tail arteries, and Figure 1d is negative control in which the primary antibody was omitted. Chronic treatment with IGF-IR AS caused a consistent reduction in IGF-IR (in terms of immunostaining intensity) compared to MM-18- or vehicle-treated tissues.

Effect of IGF-IR AS on cardiac IGF-IR expression

The expression of cardiac IGF-IR was investigated using Western blotting (Figure 2). IGF-IR AS treatment produced a small but significant reduction in the cardiac expression of IGF-IR in comparison to MM-18 ($P<0.05$, $n=3$–4)- or vehicle-treated rats ($P<0.01$, $n=4$); IGF-IR AS reduced the expression of IGF-IR to $83\pm6\%$ from $99\pm3\%$ (MM-18) or 100% (vehicle).

Effect of IGF-IR AS on resting blood pressure

There was no change in resting blood pressure over the 14-day treatment period, in any of the treatment groups. Figure 3

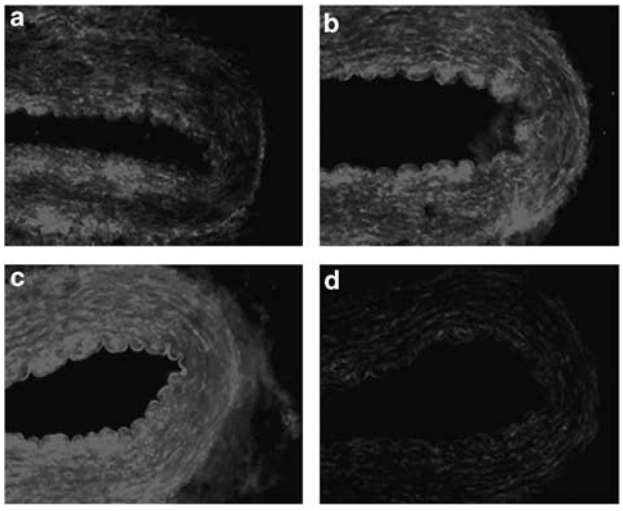

Figure 1 Representative photomicrographs showing the expression of IGF-IR in cross-section of tail arteries treated with (a) IGF-IR AS, (b) MM-18 and (c) vehicle ((d) is negative control). The immunostaining for expression of IGF-IR in the artery tissue was observed to be decreased after IGF-IR AS treatment in comparison with vehicle- or MM-18-treated tissue. Magnification $\times 400$, $n=3$.

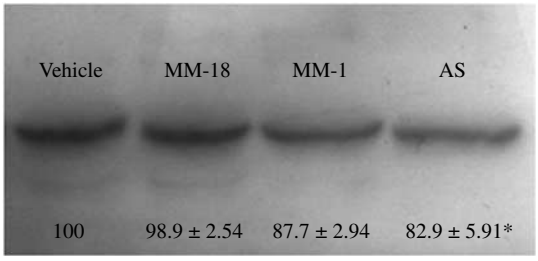

Figure 2 Western blot analysis of the effects of IGF-IR AS treatment on IGF-IR expression in cardiac muscle following 2 weeks of vehicle, MM-18, MM-1 and AS treatments (lanes 1, 2, 3 and 4, respectively, $n=3$–4). Representative results are shown, with mean $\pm$ s.e.m. of densitometric analysis relative to vehicle-treated animals shown underneath. *Indicates a significant difference to MM-18-treated animals. IGF-IR AS caused a significant reduction in the expression of both IGF-IR following 2 weeks treatment.

shows the resting systolic pressures as recorded every second day of the treatment period.

Effect of IGF-IR AS on pressor responses to AngII and NA

We chose to assess the effects of 14 days AS therapy on the two most important mediators of constrictor responses; AngII and NA, by measuring responses every second day for all treatment groups.

Figure 4 shows the effect of IGF-IR AS on pressor responses to AngII and NA in IGF-IR AS, vehicle, MM-18- and MM-1-treated rats. Comparing all treatment groups at day 14, there was a rightward shift of the dose–response curves to AngII in IGF-IR AS-treated rats ($P<0.01$, $n=6$–14) compared to vehicle (4.0-fold shift), MM-18 (4.0-fold shift, $P<0.01$) and MM-1 (4.8-fold shift, $P<0.01$) control-treated rats. Hence, the pressor response to AngII was specifically reduced by chronic administration of IGF-IR AS. The response to AngII steadily decreased over the 2 weeks of IGF-IR AS treatment period and was significantly reduced at day 14 with a 2.68 fold-rightward shift compared to day 7 ($P=0.0005$, $n=6$–14).

The effects of IGF-IR AS on NA-induced pressor responses were also examined (Figure 4b).

Chronic treatments with all three oligonucleotides significantly decreased the maximum pressor response induced by NA compared to vehicle-treated rats ($P<0.01$, $n=6$–10, Figure 4b), and thus there was no specific effect of the IGF-IR AS on responses to NA.

Effect of IGF-IR AS on cardiac and aortic AT_1R expression

The tail artery expression of AT_1R was diminished after the 2-week treatment with IGF-IR AS (Figure 5). In addition, when the effects of IGF-IR AS on cardiac AT_1R expression were assessed, a significant decrease in receptor expression was found compared to vehicle-treated tissues; cardiac AT_1R was decreased to $80\pm6\%$ in comparison of 100%; however, there was not a statistically significant difference between AS- and MM-18-treated tissues ($92\pm3\%$ of vehicle), Figure 6.

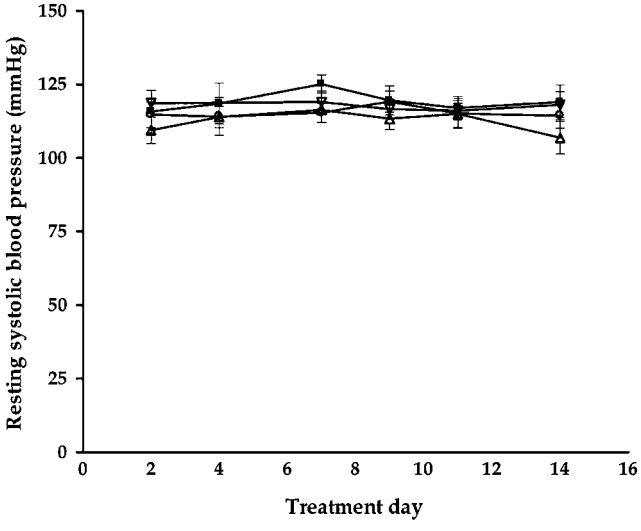

Figure 3 The effect of IGF-IR AS, MM and vehicle treatment on resting systolic pressure over the 14-day treatment period. There were no significant differences between the treatment groups, ($P>0.05$, $n=6$–14). Key: square symbols – vehicle treatment, circular symbols – AS treatment; upward triangle – full (18 base) mismatch; downward triangle – single-base mismatch.

Effect of IGF-IR AS on left ventricle : bodyweight ratio and aortic medial area

Following chronic treatment with IGF-IR AS, a reduction in left ventricle weight was seen compared to MM-18-treated rats ($P<0.05$, $n=8$). IGF-IR AS with one base mismatch also caused a decrease in LV weight ($P<0.05$, $n=8$–9). Figure 7 shows the effect of AS on left ventricle : body weight ratio. Finally, IGF-IR AS treatment resulted in a significant reduction in aortic medial cross-sectional area compared to both vehicle ($n=8$, $P<0.01$)- and MM-18-treated ($n=8$, $P<0.01$) animals, with a $27.4\pm5.7\%$ reduction compared to vehicle-treated samples (Figure 8).

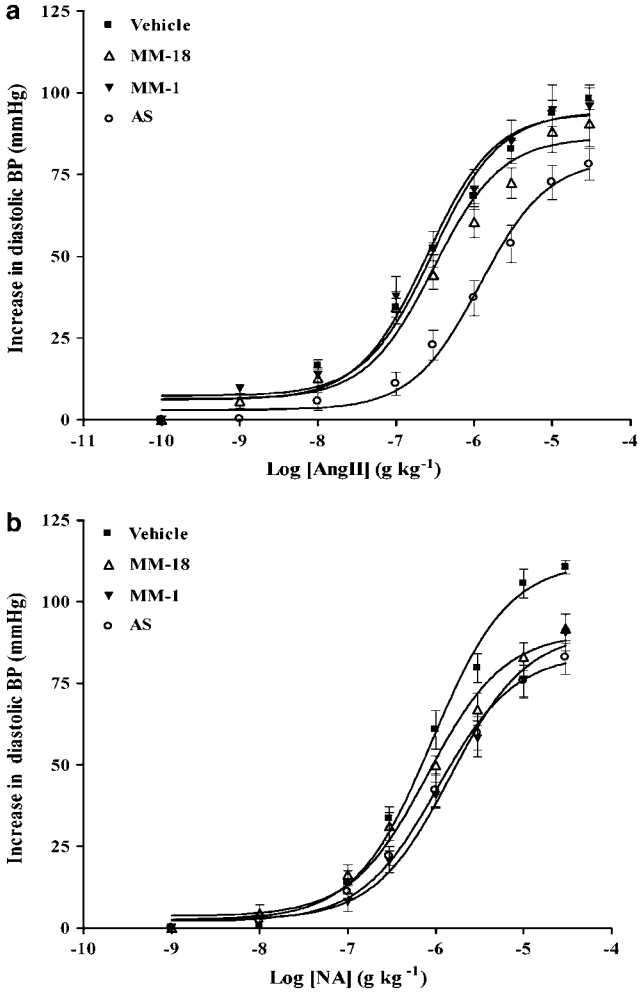

Figure 4 Effects of IGF-I receptor AS treatment on (a) pressor responses to AngII at day 14 ($n=4$–7), (b) pressor responses to NA at day 14 ($n=4$–10) compared with vehicle-, MM-18- or MM-1-treated rats. Each data point represents the mean$\pm$s.e.m. The pressor response to AngII was dramatically reduced after chronic exposure to IGF-IR AS ($P<0.001$, $n=6$–14) in comparison to vehicle or control AS MM-1 and MM-18-treated rats. AS, MM-18 or MM-1 treatment induced a significant decrease in NA E_{max} values compared with vehicle ($P<0.01$).

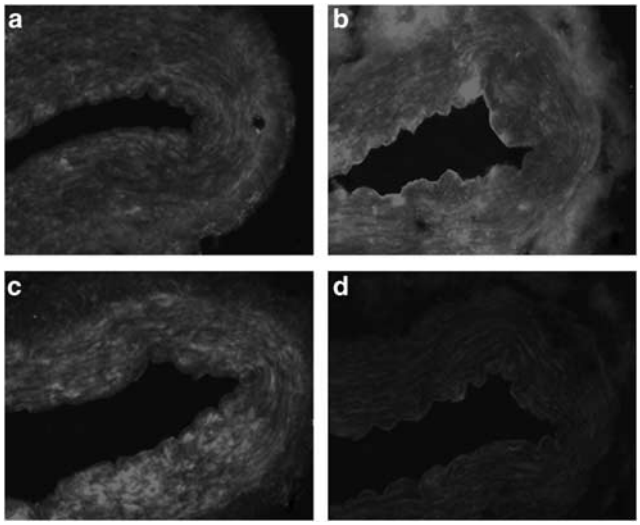

Figure 5 Representative photomicrographs showing the expression of AT_1R in cross-section of tail arteries treated with (a) IGF-IR AS, (b) MM-18 and (c) vehicle ((d) is negative control). The immunostaining for expression of AT_1R in the artery tissue was observed to be decreased after IGF-IR AS treatment in comparison to vehicle- or MM-18-treated tissue. Magnification $\times 400$, $n=3$.

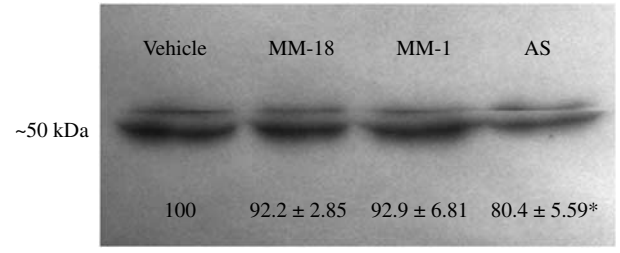

Figure 6 Western blot analysis of the effects of IGF-IR AS treatment on AT_1R expression in cardiac muscle ($n=3$–5), following 2 weeks of vehicle, MM-18, MM-1 and AS treatments (lanes 1, 2, 3 and 4, respectively). Representative results are shown, with mean ± s.e.m. of densitometric analysis relative to vehicle-treated animals shown underneath.

Discussion

The present investigation shows a specific effect of IGF-IR AS on pressor responses to angiotensin II *in vivo*, and on expression of IGF-IR and AT_1R. IGF-IR AS treatment reduced the vasoconstrictor response to AngII, and reduced the vascular and cardiac expression of IGF-IR and AT_1R.

AS treatment produced a specific reduction in the target protein. Immunostaining intensity of IGF-IR in IGF-I AS-treated aortae and tail arteries was found to be reduced compared to MM-18 or vehicle-treated tissues. In the heart, 2 weeks treatment with IGF-IR AS caused an approximately 20% reduction in the expression of cardiac IGF-IR. Thus, a systemic administration of a low dose of chimeric oligonucleotide successfully diminished the expression of the IGF-IR in cardiovascular tissues. Previous AS approaches, such as those targeting the renin–angiotensin system and β_1-adrenoceptors (Zhang *et al*., 2000; Phillips, 2002), have demonstrated a reduction in mean arterial pressure. We saw no change in resting blood pressure; it seems that the interference with cardiovascular IGF-I signalling that we produced was not sufficient to significantly alter the arterial resistance.

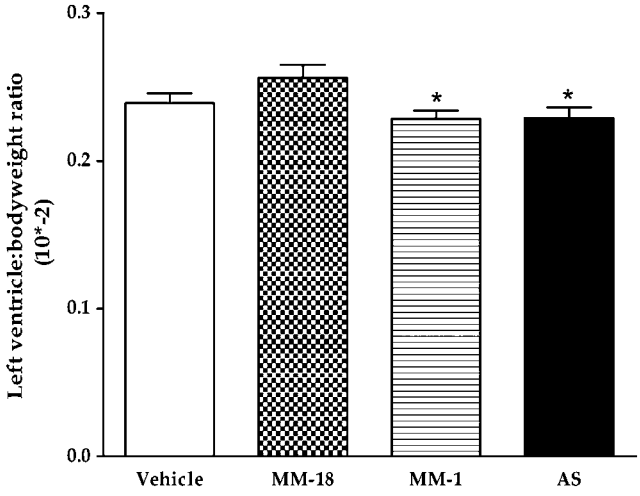

Figure 7 Effects of IGF-IR AS treatment on left ventricle (LV) to body weight (BW) ratio. Chronic treatment of IGF-IR AS caused a significant reduction in LV : BW ratio in comparison to MM-18 treated rats ($P<0.05$, $n=8$). A decrease in LV : BW ratio was also seen with one-base-mismatch administration ($P<0.05$, $n=8$–9).

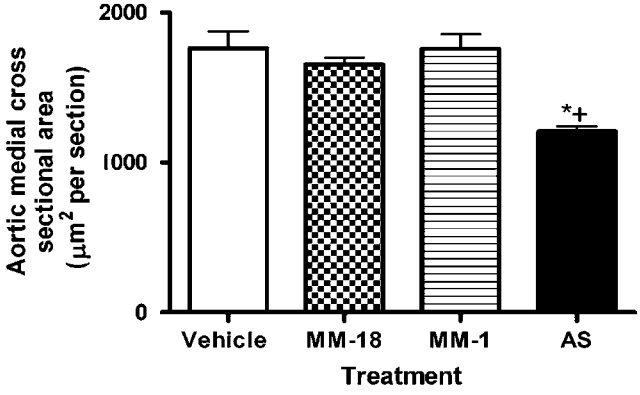

Figure 8 Quantitation of aortic medial cross-sectional area in aortae treated with vehicle, single-base mismatch (MM1), full mismatch (MM18) and IGF-IR AS for 2 weeks. $^+$Indicates a significant difference to vehicle-treated animals ($n=4$, $P<0.01$). *Indicates a significant difference to MM-18-treated animals ($n=8$, $P<0.01$).

IGF-IR AS treatment produced a specific, significant rightward shift of the AngII dose–response and caused a reduction in pressor responses induced by AngII, in comparison to vehicle-, MM-18- and MM-1 treatment. Exogenous administration of IGF-I attenuates constrictor responses to AngII and NA, *via* endothelium-dependent nitric oxide release (Walsh *et al*., 1996), and animals constitutively expressing low levels of IGF-I demonstrate elevated blood pressures (Lembo *et al*., 1996). It might have been expected, therefore, that AS knockdown of IGF-IR would cause an increase in resting blood pressure and response to vasoconstrictors. Of course, the growth effects of IGF-I on cardiac and vascular smooth myocytes will contribute to pressor rather than depressor cardiovascular effects, and in fact overexpression of IGF-I resulted in an increase in response to phenylephrine in endothelium-denuded aortae (Zhao *et al*., 2001). Our results suggest that, when IGF-I signalling is impaired, the major observable effect is a reduction in response to AngII, rather than the removal of a negative influence of IGF-I signalling on AngII constrictor responses.

In VSMC, IGF-I stimulation has been shown to potentiate AT_1R expression (Muller *et al*., 2000). Also, AngII has been found to regulate IGF-IR expression; treatment with an angiotensin-converting enzyme (ACE) inhibitor or an AngII receptor blocker decreased IGF-IR expression levels in colon tumour cells (Yasumaru *et al*., 2003), suggesting a cross-regulation between the two. The altered response to Ang II may also be attributed to the regulatory role of IGF-I in the expression of AT_1R. The effects of IGF-IR AS on expression of rat tail artery IGF-IR and AT_1R were conducted using immunohistochemistry. We observed a reduction in the expression of AT_1R after AS treatment, and not mismatch or vehicle-treated animals.

A nonspecific effect of chimeric oligonucleotide administration on the maximum response to NA was observed – with mismatch and full AS oligonucleotides producing this result. 'Off-target' effects of these agents are not uncommon; like any drug, these molecules have the potential to bind to molecules other than the intended target. We used a very low AS dose (low micrograms per injection) in order to minimise unintended effects of treatment. The dose used, 2.5 μg/injection/rat, was far lower than is typical for *in vivo* AS applications. For example, intravenous injection of doses between 0.25 and 1.0 mg day mouse^{-1} of phosphorothioate AS oligodeoxynucleotides to IGF-IR mRNA were used in Balb/c mice (Salatino *et al*., 2004) or to target c-myc gene (Leonetti *et al*., 1996).

We saw no specific or nonspecific effect of oligonucleotide treatment in pilot experiments looking at the response to acetylcholine in treated animals (no effect in any of the oligonucleotide-treated rats, data not shown), and it may therefore be that some effect on adrenergic signalling occurred in all rats treated with oligonucleotides of this chemistry. However, spontaneously hypertensive rats treated with these agents showed no nonspecific effect on response to NA (data not shown).

In the heart, administration of AngII has been seen to potentiate cardiac IGF-I and IGF-IR mRNA expression (Brink *et al*., 1999) and treatment with ACE inhibitors have been shown to inhibit cardiac IGF-I gene expression (Donohue *et al*., 1997). When investigating the regulation by IGF-I of Ang II and AT_1R expression, overexpression of IGF-I was found to blunt the upregulation of Ang II and AT_1R in diabetic heart (Kajstura *et al*., 2001). The present study examined the effect of IGF-IR AS on both cardiac IGF-I and AngII receptor expression. After 2 weeks treatment, IGF-IR AS caused an approximately 10% reduction of cardiac AT_1R compared to full-length mismatch AS. Thus, there appears to be a positive interaction between IGF-IR and AT_1R expression in this system.

In transgenic mice overexpressing IGF-I in myocardium, the total heart weight is increased by 60 and 55% increase in the number of cardiac myocytes at 210 days after birth compared to wild-type littermates (Reiss *et al*., 1996), and that IGF-I deficiency is associated with cardiac atrophy (Niebauer *et al*., 1998). We examined the effects of IGF-IR knockdown on left ventricle : body weight ratio. Chronic administration of IGF-IR AS decreased left ventricle weight. IGF-I induces its trophic effects *via* IGF-IR and that IGF-IR knockdown may contribute to the reduction in LV to body weight as seen in IGF-IR AS-treated rats. When we examined the effects of IGF-IR AS on AT_1R expression, a small reduction in AT_1R expression was also seen. AngII acts as a cardiac growth factor and is mediated *via* binding to AT_1R (Dostal & Baker, 1992; Geenen *et al*., 1993). Hence, the observed reduction in cardiac AT_1R may also contribute to the obtained reduction in left ventricle and body weight ratio followed by chronic treatment of IGF-IR AS.

Treatment with one-base mismatch IGF-IR AS produced intermediate effects on target receptor expression and on functional responses. MM-1 treatment reduced the pressor response induced by NA but not AngII, and appeared to have some effect on cardiac but not vascular IGF-IR expression. The modification of the AS sequence by one nucleotide was expected to result in an agent of intermediate efficacy; this is borne out by the results observed. Full-mismatch AS treatment had no effect on vascular or cardiac receptor expression or cardiovascular function, with the exception of a blunting of constrictor response to NA. As discussed above, this single effect of mismatch treatment may be a class effect of the AS chemistry used, as all three AS sequences used produced a decrease in the maximum response to NA. IGF-IR and mismatch AS treatments had no effects on body weight as well as water and food intake in comparison to vehicle-treated rats.

An *in vitro* vasodilatory effect of IGF-I has been shown in a number of studies. IGF-I caused relaxation of endothelin-constricted porcine coronary epicardial vessels (Hasdai *et al*., 1998), canine coronary arteries and microvessels (Oltman *et al*., 2000), and aortae of spontaneously hypertensive rats (Vecchione *et al*., 2001). *In vivo*, chronic treatment with IGF-I (3 mg kg^{-1} day^{-1}) has been found to reduce total systemic vascular resistance (Cittadini *et al*., 1996). In the present study, we examined the effect of IGF-I in vehicle-treated rats. Intravenous injection of IGF-I (1.0–500 μg kg^{-1}) did not have any effect on blood pressure. We chose not to use doses above 500 μg kg^{-1}, as these can be considered supraphysiological in the rat, whose typical serum IGF-I concentration is in the nanomolar range (Khan *et al*., 2002).

In conclusion, the results of this study suggest that a loss of IGF-IR has a predominantly inhibitory effect on vascular response to vasoconstrictor agent AngII and not NA, either through effects on downstream effects on AT_1R expression or the expression of receptors for other vasoactive signalling molecules, on changes in myocyte proliferation. It seems that, in this context, the 'tonic' haemodynamic effects of IGF-I are less important in a conscious, normotensive rat than the trophic effects on myocyte function.

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