Intravenous Insulin-like Growth Factor-I Receptor Antisense Treatment Reduces Angiotensin Receptor Expression and Function in Spontaneously Hypertensive Rats

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Received February 28, 2006; accepted June 1, 2006

ABSTRACT
The present study investigated the effects of a functional deficit in insulin-like growth factor-I signaling via chronic intravenous administration of insulin-like growth factor-I (IGF-I) receptor antisense in the conscious spontaneously hypertensive rat cardiovascular system. Insulin-like growth factor-I receptor (IGF-IR) antisense, but not full mismatch treatment, decreased IGF-IR expression in both conductance and resistance blood vessels. Aortic IGF-IR density was reduced by 67.4 ± 6.0% in antisense-treated spontaneously hypertensive rat (SHR) compared with untreated animals, whereas mismatch treatment had no effect (analysis of variance, n = 3, P < 0.01). Aortic and tail artery angiotensin II type 1 receptor expression was significantly reduced by IGF-IR antisense treatment, whereas angiotensin II type 2 receptor expression was unaffected by administration of antisense and mismatch oligonucleotides. IGF-I receptor antisense treatment caused a significant decrease in pressor responses to angiotensin II in comparison with full-length mismatch treatment (E_max was reduced to 65 ± 7 mm Hg compared with 99 ± 6 mm Hg, p < 0.05). Likewise, a reduction in pressor responses to noradrenaline was observed in hypertensive rats treated with IGF-IR antisense compared with full mismatch-treated rats (E_max was reduced to 60 ± 6 mm Hg compared with 108 ± 5 mm Hg, p < 0.01). There was no clear antisense effect on resting blood pressure and no effect at on aortic medial thickness. These results suggest that although the proliferative and vasodilator effects of IGF-I are impaired in SHR, the effects on angiotensin receptor expression remain profound.

A number of differences in the cardiovascular effects of insulin-like growth factor-I (IGF-I) have been identified in hypertensive animal models compared with their normotensive counterparts. Among the many growth-promoting effects of IGF-I is a role as a potent vascular smooth muscle cell mitogen (Du and Delafontaine, 1995), and this proliferative response is blunted in vascular smooth muscle cells (VSMC) from spontaneously hypertensive rats (SHR) (Nolan et al., 2003). An increase in IGF-I mRNA expression, however, is seen in rat models of hypertrophying aorta, portal vein, or urinary bladder (Khorsandi et al., 1992; Chen et al., 1994). IGF-I also causes direct vasodilation via a nitric oxide-dependent pathway (Pete et al., 1996; Walsh et al., 1996) and relaxes aortic rings precontracted with phenylephrine, an effect also impaired in SHR (Vecchione et al., 2001). Thus, in the vasculature, IGF-I has both pressor-inducing (VSMC mitogenic effect) and depressor effects, and both are altered in hypertensive models, whereas the expression of both the ligand and its receptor may increase in such circumstances.

There are profound interactions between IGF-I and the renin-angiotensin system with respect to vascular resistance. In VSMC, angiotensin II has been shown to potentiate IGF-I and IGF-IR expression, and this potentiation has been found to be important in the vascular growth-promoting effects of angiotensin II (Delafontaine and Lou, 1993). A similar effect of angiotensin II on IGF-I and IGF-IR gene expression was also seen in cardiac myocytes (Brink et al., 1999). Furthermore, potentiation of angiotensigen production and AT_R expression was seen in VSMC in response to IGF-I (Kamide et al., 2000; Muller et al., 2000). On the other hand, in transgenic and diabetic wild-type mice, overexpression of IGF-I was found to reduce angiotensin II and AT_R expression (Leri et al., 1999; Kajstura et al., 2001).

We recently found that IGF-IR antisense reduced the pres-
Fig. 1. Differences in the density of $^{125}$I-IGF-I binding sites in antisense-treated SHR compared with untreated or mismatch-oligonucleotide-treated aortae. Representative autoradiograms from untreated (a), mismatch-treated (b), or antisense-treated aortae (c). Nonspecific binding is shown in d. Panel e shows quantitation of binding density in the different treatment groups for SHR aortae, whereas f shows quantitation for SHR tail arteries. *, significant difference from untreated rats; +, significant difference from mismatch-treated rats (ANOVA, $P < 0.01$, $n = 3$). Scale bar = 1 mm.

Materials and Methods

Experimental Animals. The experiments were carried out using 12-week-old female SHR and age-matched Wistar Kyoto rats weighing between 160 and 200 g at the start of the experiment (SHR were purchased from Animal Resources Centre in Western Australia, Perth, Australia). We also used Hooded Wistar rats to provide a second normotensive strain to compare with the SHR. The animals were housed in North Kent Plastics (Animal Resources Center) cages with sawdust bedding and maintained on a constant 12-h light/dark cycle at 18–22°C. Animals were given normal tap water and food in the form of Clark King ARM cubes (Animal Resources Center) ad libitum. Experiments were carried out in accordance with the Australian National Health and Medical Research Council Code of Practice (1997) under a protocol approved by the Institutional Animal Ethics Committee.

Receptor Autoradiography in Spontaneously Hypertensive Rat Arteries. To quantitatively determine the effects of the treatments on IGF-IR, AT1R, and AT2R expression, autoradiography was performed on tissue sections obtained from rats treated for 4 days with 0.4 mg/kg antisense oligonucleotide, mismatch oligonucleotide, or vehicle control (four serial sections per tissue per incubation; three rats per each treatment group). IGF-IR autoradiography was performed as described previously (Sidawy et al., 1999). Frozen sections (20 μm) were incubated at room temperature for 2 h in 50 mM Tris-HCl buffer with 0.1% bovine serum albumin, 1 mg/ml bacitracin, and 10 mM MgCl2 containing 40 pM $^{125}$I-IGF-I (Auspep, Parkville, VIC Australia; iodination performed by ProSearch, Sydney, NSW, Australia). Nonspecific binding was determined by incubating sequential tissue sections in buffer containing radioligand and excess unlabeled IGF-I (0.1 μM). Angiotensin receptor autoradiography was conducted as described previously (McDougall et al., 2000) using buffer containing 50 nM Sar1-Ile8-125I angiotensin II (Auspep, Parkville, VIC Australia; iodination performed by ProSearch); AT1 receptor levels were determined using the addition of the AT1R antagonist PD123319 (10 μM; Sigma, Castle Hill, NSW, Australia), whereas AT2R levels were determined using the AT2R antagonist losartan (10 μM; DuPont, Wilmington, DE); nonspecific binding was determined in the presence of 20 μM angiotensin II (Auspep). Sections were incubated in the above solutions for 60 min followed by four successive 3-min washes in ice-cold buffer. After incubations, sections were washed in ice-cold buffer (2 × 2 min), dried overnight, and exposed to Kodak Biomax MR film (Eastman Kodak, Rochester, NY) for 5 days. Optical densities were determined using a computer-assisted digitization system (Scion Corporation, Frederick, MD) and were converted to disintegrations per millimeter² via the use of ¹⁴C microscale standards. A low level of nonspecific binding was consistent across the different treatment groups and was subtracted from total binding. Nonspecific binding was therefore subtracted from total binding to determine specific binding densities for both IGF-IR and angiotensin receptor binding assays.

Surgical Procedure. Rats were anesthetized 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 heparinized saline solution (100 U ml⁻¹). The cannulas were tunneled subcutaneously to exit at the back of the neck.

In Vivo Effects of Antisense Treatments. Rats received one of the following treatments via the jugular vein cannula: 1) antisense oligonucleotide targeting the IGF-IR (antisense: 5'-UCC-CAC-AGC-TGC-UGC-AAG-3' with a modification of 1-6 2'OMe RNA, 7-12
IGF-IR Antisense Treatment Specifically Reduced Aortic and Tail Artery IGF-IR Expression in Spontaneously Hypertensive Rats. The IGF-IR expression levels in the aortae and tail artery of IGF-IR antisense and control-treated SHR were examined using receptor autoradiography. Specific $^{125}$I-IGF-I binding was consistently observed in the vessel wall in untreated SHR, and there was no observable variation in the binding density from the lumen to the adventitia. In preliminary experiments, the level of specific binding remaining in the presence of des-(1–3)-IGF-I, a truncated form of IGF-I that does not bind with high affinity to IGF-I binding proteins, was less than 15% of the total specific binding.

IGF-IR antisense treatment resulted in a significant, specific reduction in IGF-IR levels in both conductance and resistance vessels. Aortic IGF-IR density was reduced by 67.4 ± 6.0% in antisense-treated SHR compared with untreated animals, whereas mismatch treatment had no effect (ANOVA, n = 3, P < 0.01 comparing antisense-treated aortae with both untreated and mismatch-treated aortae; Fig. 1e). In tail arteries, antisense treatment resulted in a 52.9 ± 3.0% reduction in IGF-I binding compared with untreated vessels; mismatch treatment again had no effect (ANOVA, n = 3, P < 0.01 comparing antisense-treated tail arteries to both untreated and mismatch-treated tail arteries; Fig. 1f).

IGF-IR Antisense Treatment Reduced AT$_1$R Expression in SHR. AT$_1$R expression (determined in the presence of the AT$_1$R ligand PD123319) was 2- to 3-fold greater than AT$_2$R expression (determined in the presence of the AT$_2$R antagonist losartan) in untreated SHR vessels. AT$_1$R expression was significantly reduced by IGF-IR antisense treatment (Fig. 2). IGF-IR antisense treatment significantly reduced AT$_1$R binding in both aorta (52.2 ± 8.9% reduction in AT$_1$R binding compared with untreated rats; ANOVA n = 3, P < 0.01 comparing antisense-treated aortae with both untreated and mismatch-treated aortae) and tail arteries (48.1% reduction in AT$_1$R binding compared with untreated rats; ANOVA n = 3, P < 0.01 compared with untreated tail arteries). Mismatch treatment resulted in a trend toward reduction in AT$_1$R expression; however, there was no significant difference in expression between mismatch and untreated animals (ANOVA, n = 3, P = 0.09). There were no significant effects on AT$_2$R levels in either antisense or mismatch.
match oligonucleotide-treated animals compared with aortae or tail arteries from untreated animals (ANOVA, \( n = 3, P > 0.05 \)).

**IGF-IR Antisense Reduced Responses to Vasoconstrictor Agents, Angiotensin II, and Noradrenaline in Spontaneously Hypertensive Rats.** The in vivo effects of IGF-IR antisense on vascular responses to angiotensin II and noradrenaline in SHR were investigated over 14 days. Figure 3 illustrates that although there were no changes in responses after 7 days of treatment (Fig. 3, a and b), administration of IGF-IR antisense produced a significant reduction in the maximum response to angiotensin II at 14 days (Fig. 3c, \( E_{\text{max}} \) was reduced to 65 ± 7 mm Hg compared with 99 ± 6 mm Hg for mismatch-treated rats, \( p < 0.05, n = 4–8 \)) and noradrenaline (Fig. 3d, \( E_{\text{max}} \) was reduced to 60 ± 6 mm Hg compared with 108 ± 5 mm Hg for mismatch-treated rats, \( p < 0.01, n = 4–8 \)).

**IGF-IR Antisense Treatment Had No Significant Effect on Aortic Medial Cross-Sectional Area or Left Ventricle/Body Weight Ratio.** Figure 4 shows the effect of IGF-IR antisense treatment on SHR left ventricle/body weight ratio (Fig. 4a) and heart/body weight ratio (Fig. 4b). Although it does seem that there is a trend toward a reduction in cardiac parameters in the IGF-IR antisense-treated group, there were no significant differences between the treatment groups (\( n = 4–5, p > 0.05 \)). Figure 4c shows that antisense treatment had no effect on aortic medial cross-sectional area.

**The Specific Reduction in Response to Angiotensin II and Noradrenaline after IGF-IR Antisense Was Greater in SHR than Normotensive Rats.** We then compared the pressor dose-response curves with angiotensin II and noradrenaline for all three rat strains: Hooded Wistar, Wistar Kyoto, and SHR (Fig. 5). Antisense treatment had no effect on \( E_{\text{max}} \) or \( E_{50} \) values for the pressor response to noradrenaline or angiotensin II in Wistar Kyoto rats. In Hooded Wistar rats, antisense treatment produced a 21.5 ± 4.5% reduction in the \( E_{\text{max}} \) for noradrenaline compared with vehicle-treated rats (ANOVA, \( n = 6, P < 0.001 \)) and a 9.8-fold decrease in potency for angiotensin II compared with vehicle-treated rats. In SHR, the maximum responses to both noradrenaline and angiotensin II and noradrenaline were greatly reduced by antisense treatment, by 34 ± 4.5 and 44 ± 5.9%, respectively. When we converted the vehicle-treated data for each strain to 100%, there was a significantly greater effect of antisense treatment on \( E_{\text{max}} \) responses to both angiotensin II and noradrenaline in SHR than in Hooded Wistar or Wistar Kyoto rats (ANOVA, \( p < 0.01, n = 6 \)). There was no effect of the control (full mismatch) oligonucleotide on the potency or maximum efficacy of noradrenaline or angiotensin II in any of the strains examined (ANOVA, \( n = 4–6, P > 0.05 \)).

**IGF-IR Antisense Treatment Had No Major Effect on Resting Blood Pressure.** The effect of chronic administration of IGF-IR antisense on blood pressure is shown in Fig. 6. In Hooded Wistar rats, neither IGF-IR antisense treatment nor mismatch control had any effect on resting blood pressure (Fig. 6a) nor was there any effect in Wistar Kyoto rats (data not shown). IGF-IR antisense-treated rats exhibited lower basal blood pressure after 14 days treatment than mismatch- or vehicle-treated spontaneously hypertensive rats; systolic pressure was 147 ± 5 mm Hg compared with 187 ± 25 or 182 ± 11 mm Hg, respectively (Fig. 6b, \( p < 0.05, n = 6 \)). However, this was the only time point at which a significant difference was evident, and at day 11, the vehicle and antisense groups had almost identical resting blood press-
sures. Treatment with IGF-IR antisense has no effect on heart rate of hypertensive rats over 14 days (data not show).

Discussion

In the present study, chronic intravenous administration of IGF-IR antisense in spontaneously hypertensive rats produced a profound reduction in responses to vasoconstrictor agents angiotensin II and noradrenaline, lowered resting blood pressure more than relevant controls, and reduced the vascular expression of IGF-IR and AT\textsubscript{1}R. These effects were greater than those observed in normotensive rats, where a smaller reduction (or none) was observed.

IGF-IR antisense treatment produced a specific reduction in the binding of \textsuperscript{125}I-IGF-I to both aortae and tail arteries of treated animals, whereas the mismatch-control oligonucleotide had no effect. Thus, systemic administration of chimeric oligonucleotide successfully diminished the expression of the IGF-IR in vasculature of SHR. The level of reduction in target expression is significant, particularly because we used microgram per kilogram doses rather than milligram per
indeed a significant difference between AT1R levels in anti-

have revealed as statistically significant. However, there was

AT1R levels in this study, which a greater sample size could

gree of nonspecific effect of oligonucleotide treatment on

untreated rats. Thus, there may have been some small de-

larly amenable to antisense intervention.

vascular AT1R (but not AT2R) expression in these animals.

A reduction in IGF-IR seemed to result in a reduction in

vascular AT,R (but not AT,R) expression in these animals.

angiotensin II binding in the presence of

PD123319, but not losartan, was reduced in antisense-
treated rats. Antisense oligonucleotides do have nonse-
quence-specific and off-target effects, and in the current
study, AT,R levels were lower in mismatch-treated rats than
untreated rats. Thus, there may have been some small de-
gree of nonspecific effect of oligonucleotide treatment on

AT,R levels in this study, which a greater sample size could
have revealed as statistically significant. However, there was
indeed a significant difference between AT,R levels in anti-
sense-treated rats compared with both forms of control—
untreated and mismatch-treated rats. We can therefore con-
clude that IGF-I receptor knockdown does reduce AT1
receptor levels compared with relevant controls.

We were unable to accurately determine whether there
was a greater antisense-mediated reduction in AT1 receptor
levels in SHR compared with normotensive strains. The
trend toward a reduction in AT1 receptor expression in the
mismatch control oligonucleotide-treated SHR made a quan-
titative comparison invalid.

This reduction in the density of the angiotensin receptors
responsible for vasoconstrictor effects of angiotensin II
seemed to have important functional consequences in the

treated animals. IGF-IR antisense treatment (and not mis-
match or vehicle treatment) produced a significant reduction
in vascular response to angiotensin II in SHR. The deficit in
both AT,R density and in the constrictor effects of angioten-
sin after IGF-IR antisense treatment may provide insight
into the functional relevance of previous work indicating that
IGF-I acts to increase AT,R expression in vascular smooth
muscle cells at the transcriptional level (Muller et al., 2000).

The inhibitory effect of IGF-IR antisense on angiotensin II
responses observed in this study may be due to the loss of the
stimulatory effects of IGF-I in the expression of AT,R. These
data suggest that there may be a powerful and ongoing role
in SHR for IGF-I stimulation of AT,R expression. Despite
evidence that AT,R expression is increased by IGF-I in vas-
cular smooth muscle cells (Kambayashi et al., 1996), we saw
no effect of IGF-IR antisense treatment on AT,R binding; we
are currently investigating whether this is due to alterations
in the effects of IGF-I on receptor expression in SHR.

Changes in the level of radioligand binding to both the IGF-I
receptor and AT receptors could reflect altered affinity rather
than receptor number. However, in previous studies using
antisense targeting the same region of the IGF-I receptor
mRNA, we have exclusively observed changes in receptor
number and not affinity (Wraight et al., 2000).

In the present study, we also found a reduction in vascular
responses to noradrenaline in SHR treated with IGF-IR an-
tisense. IGF-I has been found to up-regulate VSMC α1-adre-
noceptor expression (Hu et al., 1996). The activities of IGF-I
are predominantly mediated through binding to the IGF-IR;

hence, a decrease in aortic IGF-I expression induced by
IGF-IR antisense would probably attenuate any effect of
IGF-I on α1-adrenoceptor expression, which may contribute
to the in vivo decrease in noradrenaline response. The focus
of this study was the interaction between IGF-I and angio-
tensin receptor function; however, we are currently investi-
gating the effects of IGF-IR antisense on other signaling
pathways in vitro using tissues from antisense-treated ani-
mals.

It might be expected that IGF-IR antisense treatment
would increase resting blood pressure, since IGF-I causes
nitric oxide release (Fryburg, 1996; Pete et al., 1996; Walsh
et al., 1996), and blood pressure has been shown to be ele-
vated in IGF-I knockout mice (Tivesten et al., 2002). We saw
no major change in basal blood pressure during the 14-day
antisense treatment period in any of the rat strains. SHR
blood pressure was significantly lower at one time point
(after 14 days) in the antisense-treated rats than the mis-
match or vehicle controls; however, this was mainly due to a
rise in the vehicle-treated rats pressure from day 11 to day
14. The lack of increased pressure in IGF-IR antisense-
treated animals may be due to the blunting of IGF-I effects

Fig. 6. Effects of IGF-I receptor antisense on resting systolic blood pressure (BP). Each data point represents the mean ± S.E.M. There was no
effect of antisense treatment on blood pressure in Hooded Wistar rats (a). IGF-IR antisense significantly reduced blood pressure at day 14 (p <
0.05, n = 6) compared with mismatch-treated SHR (b).

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on vascular resistance in hypertensive animals (Vecchione et al., 2001; McCallum et al., 2005), or perhaps this simply reflects the relatively minor role of IGF-I in regulation of vascular resistance—blood pressure only drops 5 to 10 mm Hg after IGF-I administration in rats (Walsh et al., 1996; P. J. White, unpublished observations).

Although we observed a significant reduction in the vascular medial cross-sectional area in Hooded Wistar rats (Nguyen and White, 2005), there was no such effect in SHR in the present study. As mentioned above, the effects of IGF-I are altered in SHR, and this applies to the proliferative effects as well as those related to vascular resistance. Nolan et al. (2003) found that there was an impairment in IGF-I-induced proliferation in aortic vascular smooth muscle cells from SHR compared with Wistar Kyoto control, and our observations support this—we found that although IGF-IR expression was profoundly reduced, there was no consequent change in vascular thickness in SHR in vivo. Because vascular structure was unaffected in IGF-IR antisense-treated SHR, we suggest that the observed decrease in vasoconstrictor responses to angiotensin II may be due to changes in AT1 receptor expression rather than changes in tissue contractility in these animals.

We saw a greater inhibitory effect of AS treatment on the response to angiotensin II in particular and noradrenaline to a lesser extent in SHR compared with Hooded Wistar (where a moderate reduction in potency was observed) and Wistar Kyoto rats (where no effect was observed). It is possible that the efficacy of the antisense was greater in SHR than normotensive rats and that this is the reason for the greater effects of antisense treatment on responses to angiotensin II. We consider this unlikely, however, given that we find a moderate reduction in potency in SHR in vivo. Because vasculature was unaffected in IGF-IR antisense-treated SHR, we suggest that the observed decrease in vasoconstrictor responses to angiotensin II may be due to changes in AT1 receptor expression rather than changes in tissue contractility in these animals.

Therefore, the greater antisense effect is likely to be due to a greater involvement of IGF-I in these responses in SHR. Both renin-angiotensin-aldosterone system activity and sympathetic nervous system activity are elevated in SHR (Grisk, 2005), and therefore, the efficacy of the antisense may have been greater in rats where these systems are more "basally" active.

The results of this study show that IGF-IR knockdown induces a profound inhibitory effect on vascular response to vasoconstrictor agents, possibly through effects on AT1 receptor signaling, alone or in combination with downstream effects on receptors for other vasoactive signaling molecules in the SHR vascular system. We are currently investigating these possibilities.

Acknowledgments

We thank Dr. Andrew Lawrence and Cameron Adams (Howard Florey Institute for Medical Research, Parkville, VIC, Australia) for assistance with the receptor autoradiography experiments.

References


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