Impaired Insulin-Like Growth Factor I Vasorelaxant Effects in Hypertension

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Abstract—Insulin-like growth factor I (IGF-I) can be considered a factor potentially involved in arterial hypertension not only for its growth-promoting features but also for its effects on vascular tone. Nevertheless, the actions of the hormone on vascular reactivity are still unexplored in hypertension. Therefore, the vasodilation induced by increasing doses of IGF-I and the modulation of norepinephrine vasoconstriction induced by low levels of the hormone were tested on aortic rings of spontaneously hypertensive and normotensive rats. The results indicate that the vasodilation evoked by IGF-I is impaired in hypertensive rats (Δ% of maximal vasorelaxation, 30±1 versus 41±1; P<0.01), and after the removal of endothelium or the inhibition of endothelial NO synthase, the vasodilation evoked by the hormone was blunted in both rat strains and became similar between hypertensive and normotensive rats (Δ% of maximal vasorelaxation, 21±1 versus 20±1; P=NS). Moreover, IGF-I does not show any effect on norepinephrine vasoconstriction in hypertensive rats, and this alteration may depend on the lack of sensitizing effect exerted by IGF-I on α1-adrenergic–evoked NO vasorelaxation. The defect in IGF-I vascular action is also present in young spontaneously hypertensive rats (age 5 weeks). In conclusion, our data demonstrate that IGF-I vasorelaxant properties are impaired in spontaneously hypertensive rats, suggesting that such defect may play a causative or permissive role in the development of hypertensive conditions. (Hypertension. 2001;37:1480-1485.)

Key Words: vascular reactivity ■ aorta ■ norepinephrine ■ nitric oxide ■ receptors, adrenergic, alpha

Insulin-like growth factor I (IGF-I) is a circulating and locally produced protein that stimulates growth, differentiation, and metabolism of a wide variety of cell types. Several studies suggest that IGF-I plays an important role in the cardiovascular system. In particular, it has been shown that IGF-I is a critical determinant of vascular growth responses to several stimuli both in vivo and in vitro, representing a convergence point of multiple neurohumoral factors.

There is increasing evidence that IGF-I, in addition to its mitogenic effects, also acts on vascular tone. Several in vivo studies have reported that IGF-I has vasorelaxant properties, and such evidence is also supported by studies performed in more elementary models of vascular function, such as aortic rings. In particular, in the presence of low levels of IGF-I, the contraction induced by norepinephrine is markedly blunted, and this effect is abolished by endothelial denudation or by treatment with NO inhibitor, demonstrating that IGF-I acts on vascular tone through an endothelial NO release. Furthermore, it has been demonstrated that higher doses of IGF-I are able to evoke a net vasodilation in coronary artery, and this effect depends on both endothelial and smooth muscle components. It is also particularly intriguing that gene-targeted mice, which have partially ablated IGF-I, show an increase in mean arterial pressure, suggesting that the action of IGF-I on vascular tone may contribute to blood pressure homeostasis. Therefore, IGF-I can be considered a factor potentially involved in arterial hypertension not only for its growth-promoting features, affecting the remodeling of cardiovascular system, but also for its effects on vascular tone. Nevertheless, the actions of the hormone on vascular reactivity are still unexplored in hypertension.

To clarify this issue, we examined the effects of IGF-I on vascular function of vessels from spontaneously hypertensive rats (SHR), a rat model of genetic hypertension, and from Wistar-Kyoto rats (WKY), the reference normotensive rat strain. In particular, we examined both the vasodilation induced by high doses of IGF-I and the modulation of norepinephrine vasoconstriction induced by low levels of the hormone. Moreover, we analyzed the mechanisms accounting for IGF-I vascular effects in hypertensive conditions.

Methods

Experimental Animals

The studies were conducted in 85 WKY and 51 SHR (Charles River Laboratory) age 12 to 14 weeks and in 16 WKY and 18 SHR age 5

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weeks. The animals were kept in a temperature-controlled room (23°C to 25°C) with a 12-hour light/dark cycle. Food and water were provided ad libitum. Experiments were performed after acclimatization to the housing condition for at least 1 week. Systolic blood pressure was measured in conscious rats by tail-cuff plethysmography (PE-300, Narco Biosystems Inc) and recorded on a multichannel polygraph (Universal Oscillograph, Harvard Instruments). The experimental protocol was in accordance with our institutional guidelines for animal research.

**Studies on Aortic Rings**

Rats were weighed and decapitated. Thoracic aorta was dissected and placed in cold Krebs-Henseleit buffer (in mmol/L): NaCl 118.3, KCl 4.7, CaCl₂ 2.5, MgSO₄·7H₂O 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, and glucose 5.6). The aorta was cleaned of the adhering perivascular tissue and cut into 3-mm-long rings. Aortic rings were suspended in isolated tissue baths filled with 20 mL Krebs’ buffer continuously bubbled with 5% CO₂/95% O₂ (pH 7.37 to 7.42) at 37°C. One end of the aortic ring was connected to a tissue holder; the other, to an isometric force transducer. The signal was passed to a Gould pressure processor and then acquired in a computerized system by DASA (Data Acquisition and Signal Analysis) (Gould). Generated curves were analyzed by View II software (Gould Instruments) with a sensitivity of 5 mV/mm. Constrictions to different agonists such as norepinephrine or endothelin were evaluated in both rat groups. In particular, at the end of the aortic ring was connected to a tissue holder; the other, to an isometric force transducer. The signal was passed to a Gould pressure processor and then acquired in a computerized system by DASA (Data Acquisition and Signal Analysis) (Gould). Generated curves were analyzed by View II software (Gould Instruments) with a sensitivity of 5 mV/mm. Constrictions to different agonists such as norepinephrine or endothelin were evaluated in both rat groups. In particular, at the end of the experiment.

In the first experimental series, the effect of IGF-I on vascular function in young SHR and WKY (age 5 weeks) was tested by dose-response curves to phenylephrine (10⁻⁶ mol/L). Rings were equilibrated for 90 minutes in the unstrained condition. The length of the smooth muscle was increased stepwise to adjust passive wall tension to 1.5 g for 5-week-old rats and 2.0 g for 12- to 14-week-old rats. This tension was found optimal for contractions of aorta from WKY and SHR by testing the contractions to norepinephrine (10⁻⁶ mol/L). Care was taken to avoid vessel damage, and the vascular function was tested by dose-response curves to phenylephrine (10⁻⁶ to 10⁻⁴ mol/L). The signal was passed to a Gould pressure processor and then acquired in a computerized system by DASA (Data Acquisition and Signal Analysis) (Gould). Generated curves were analyzed by View II software (Gould Instruments) with a sensitivity of 5 mV/mm. Constrictions to different agonists such as norepinephrine or endothelin were evaluated in both rat groups. In particular, at the end of the experiment.

The following drugs were used: IGF-I (human recombinant), norepinephrine, 8-hydroxy-1-nitro-1-arginine methyl ester (L-NAME), adenosine diphosphate, ionomycin, endothelin, phenylephrine, KCl (Sigma Chemical Co), and UK 14,304 (Research Biochemicals International). Drugs were prepared daily in distilled water, except ionomycin, which was dissolved in dimethyl sulfoxide (Sigma Chemical Co). Full dose-response curves were obtained for each agent. Contractile responses were evaluated as milligrams of tension, and the maximal contraction was considered baseline when subsequent vasorelaxations were evoked. Vasorelaxations were expressed as percent reduction in contraction (the maximal vasorelaxation attained with papaverine was 100% vasorelaxation). In the first experimental series, the vasodilatation evoked by increasing doses of IGF-I (50 to 500 nmol/L) in aortic rings of both WKY and SHR preconstricted with phenylephrine (10⁻⁶ mol/L) or KCl (40 nmol/L) was evaluated. IGF-I vascular action was also tested after endothelium removal or after exposure to the NO synthase inhibitor L-NAME (300 μmol/L for 15 minutes).

In the second experimental series, the effect of IGF-I on vasoconstriction evoked by different agonists such as norepinephrine or endothelin was evaluated in both rat groups. In particular, at the end of the equilibration period we added to aortic rings increasing doses of norepinephrine (10⁻⁶ to 10⁻⁴ mol/L) or endothelin (10⁻⁶ to 10⁻⁴ mol/L) before and after 30 minutes of preincubation with low levels of IGF-I (50 nmol/L). The effect of IGF-I was also examined after incubation with L-NAME.

In the third experimental series, we explored whether the vascular action evoked by low levels of IGF-I could be related to a generalized sensitization of endothelium NO-mediated responses. In particular, receptor (acetylecholine 10⁻⁶ to 10⁻⁴ mol/L; adenosine diphosphate 10⁻⁶ to 10⁻⁴ mol/L; a selective α₁-adrenergic agonist, UK 14,304 10⁻⁶ to 10⁻⁴ mol/L) and nonreceptor (ionomycin 10⁻⁶ to 10⁻⁴ mol/L) endothelium NO-mediated vasodilations were tested in control conditions and after 30 minutes of exposure to IGF-I. Finally, in the last experimental series, we tested the effects of IGF-I on vascular function in young SHR and WKY (age 5 weeks).

**Evaluation of IGF-I Receptors on Aortic Tissue of WKY and SHR**

Aortas were removed and immediately homogenized at 4°C in Tris-HCl buffer (20 mmol/L, pH 7.4) containing 10% sucrose. Homogenates were sequentially centrifuged at 1500g for 20 minutes, and the resulting supernatants were centrifuged at 20,000g to obtain the P2 fractions. Pellets, corresponding to plasmatic membranes, were resuspended in ice-cold lysis buffer (50 mmol/L Tris [pH 7.4], 150 mmol/L NaCl, 1 mmol/L EDTA, 0.25% Na-deoxycholate, 1% NP-40, 1 mmol/L PMSF, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin). An aliquot of protein was used for protein determination, and the remainder was diluted 1:1 with SDS–bromphenol blue reducing buffer (14.4 mmol/L 2-β-mercaptoethanol, 60 mmol/L Tris [pH 6.8], 2% SDS, 0.1% bromphenol blue, 25% glycerol) heated in boiling water for 5 minutes. Aliquots (35 μg of proteins) were subjected to SDS-PAGE electrophoresis and were run with the use of 8% SDS polyacrylamide gels on a minigel apparatus (Biorad, Mini Protean II Cell); gels were electroblotted on nitrocellulose membranes, which were blocked overnight in milk. Blots were then incubated for 1 hour at room temperature with IGF-I-β-chain receptor (IGF-I-BR) pAb (1 μg/mL), washed, and incubated for 1 hour with secondary antibodies (peroxidase-coupled anti-rabbit, Amersham) diluted 1:10 000 in T-TBS. Immunostaining was revealed by ECL (Amersham).

**Statistical Analysis**

The results are expressed as mean±SEM. Student’s t test, ANOVA, or repeated-measures ANOVA followed by Bonferroni’s test were used when appropriate. A 2-tailed value of P<0.05 was considered significant.

**Results**

As expected, systolic blood pressure was significantly higher in 12- to 14-week-old SHR than in WKY (180±2 versus 122±2 mm Hg; P<0.01), whereas it was similar in young rats (120±3 versus 118±2 mm Hg; P=NS). Cumulative addition of phenylephrine caused contractions in all aortic segments, revealing a good general responsiveness of the vessels used for the further investigations. L-NAME exposure slightly increased basal vascular tone, and this vascular effect was comparable between the 2 rat strains (data not shown). Acetylcholine evoked a reduced vasodilation in SHR compared with WKY in both old (Δ% relaxation, from 5±1 to 54±2 versus from 7±1 to 78±2; P<0.01) and young rats (Δ% relaxation, from 4±1 to 50±1 versus from 5±1 to 76±2; P<0.01). In contrast, sodium nitroprusside evoked a similar vasodilation between SHR and WKY (data not shown).

**Effects of IGF-I on Aortic Rings of WKY and SHR, Preconstricted With Phenylephrine or KCl**

Phenylephrine induced a similar contraction in both WKY and SHR (maximal vasostenosis, 1425±81 versus 1389±99 mg; n=18; P=NS). As shown in Figure 1, IGF-I evoked a dose-dependent vasodilation in aortic rings of both rat strains. The vascular response evoked by the hormone, however, was significantly reduced in SHR compared with WKY. L-NAME exposure blunted the IGF-I vasodilation in vessels of WKY and SHR compared with control conditions, and, more importantly, in these experimental conditions the vasodilation evoked by IGF-I became similar between the 2 rat strains. Furthermore, IGF-I–evoked vasodilation was also blunted in endothelium-denuded aortic rings compared with that observed in intact aortic rings in both WKY (Δ% of
Effects of IGF-I Exposure on Vasoconstriction Evoked by Norepinephrine and Endothelin in WKY and SHR

As shown in Figure 2, norepinephrine evoked a greater dose-dependent vasoconstriction in SHR than in WKY. IGF-I exposure blunted the vasoconstriction evoked by norepinephrine in WKY, and this vasorelaxant effect of IGF-I was absent in SHR. L-NAME administration abolished the IGF-I attenuation of norepinephrine vasoconstriction in WKY (n=7; data not shown). Endothelin-evoked vasoconstriction was slightly higher in hypertensive rats than in normotensive rats (maximal vasoconstriction, 1646±39 mg; n=12; P=0.07), and IGF-I exposure did not affect this vasoconstriction in either rat strain (∆%, −2±1 versus 1±1; P=NS).

Evaluation of IGF-I Receptors on Aortic Tissue of WKY and SHR

Western immunoblotting with IGF-IβR antibody revealed a single band to 95 kDa corresponding to the β-subunit of IGF-IβR.
IGF-I receptor. Immunoblot revealed that IGF-I receptor is equally expressed in the plasmatic membranes of aortic tissue of WKY and SHR (Figure 5).

**Discussion**

Our data demonstrate that SHR are less sensitive to the vasorelaxant effects evoked by IGF-I than normotensive rats. In particular, SHR show a reduced vasodilation to increasing doses of IGF-I and an impaired IGF-I attenuation of norepinephrine-induced vasoconstriction compared with that observed in WKY. The defect of IGF-I vascular action in SHR preexists the development of hypertension and depends on the impairment of the endothelial NO component involved in the vasorelaxant effects of the hormone.

It is well known that IGF-I has a profound impact on the vascular system. Actually, IGF-I is produced by endothelial and vascular smooth muscle cells, and specific receptors for IGF-I are abundant in the vascular wall.17–19 Furthermore, IGF-I plays an important role in the intrinsic growth program of the blood vessels, participating in the vascular remodeling in several cardiovascular diseases such as arterial hypertension.20–22 This latter pathological condition is characterized by both structural vascular adaptations and inadequate regulation of vascular tone to the increased hemodynamic load. Thus, the vascular system in hypertension is challenged by structural and functional changes, which contribute to sustain the increased blood pressure levels. In recent years, it has become clear that IGF-I not only contributes to vascular remodeling but also exerts important effects on vascular tone.10–13 Thus far, no data are available on the vasorelaxant effects of IGF-I in hypertensive conditions. The results of the present study clearly demonstrate that IGF-I effects on vascular function are impaired in hypertension. In particular, the vasodilation induced by high levels of IGF-I, a condition simulating the effect of IGF-I locally in the vasculature as autocrine and paracrine factor, is reduced in SHR compared with that observed in WKY. Hypertensive rats are also defective in the attenuation of norepinephrine vasoconstriction realized by lower IGF-I levels, observed in the normotensive rat strain. Thus, the vasorelaxant effects realized by both low and high levels of IGF-I are altered in SHR. Furthermore, the observation that the impaired response to IGF-I in SHR is present before the onset of hypertension indicates that the alterations of vascular reactivity to the hormone are not acquired with the hypertensive condition and seem to be a trait of the hypertensive genetic background.

The reduced IGF-I responsiveness in SHR is not related to the difference in IGF-I receptors on vascular tissue between hypertensive and normotensive rats. Our receptor analysis, however, cannot exclude a difference in IGF-I receptor binding or affinity between the 2 rat strains. Because several studies have demonstrated that both endothelial NO12,13,23 and potassium channels mediate IGF-I vascular action,14,15,24 we extended our observations to characterize the role of these components in the abnormal vascular response to IGF-I in hypertensive rats. In regard to this issue, it is important to emphasize that vasorelaxation evoked by NO is also realized by smooth muscle potassium channels.25–27 Thus, potassium channels represent the converging point of IGF-I NO-dependent and -independent vasorelaxations. Because KCl exposure abolished IGF-I–evoked vasodilation in aortic rings of both rat strains, we cannot examine whether differences in the IGF-I vascular effect in hypertension are attributable to a defect in potassium channels. Our findings are supported by a recent observation that IGF-I vasodilation in conduit vessels is attenuated by L-NAME and completely blocked by KCl.24 During the removal of endothelium or the inhibition of
endothelial NO release, however, IGF-I evoked a similar vasodilation between normotensive and hypertensive rats, indicating that impairment of the endothelial NO component plays a key role in the abnormal IGF-I vasodilation observed in hypertensive rats. The defect in NO component may be ascribed to an altered NO metabolism or an altered sensitivity of smooth muscle. Because exogenous NO donor, which also acts through smooth muscle potassium channels, evokes a similar vasodilation in both hypertensive and normotensive rats, we can speculate that the defect in IGF-I vasodilation observed in SHR must be ascribed to impaired NO signaling upstream of the activation of potassium channels. Furthermore, the selective attenuation of norepinephrine vasoconstriction by IGF-I is dependent on an endothelial NO mechanism, suggesting that the impairment of this IGF-I vascular action in SHR recognizes an endothelial NO dysfunction. Our findings are strongly supported by other recent data in SHR, which have demonstrated an impaired IGF-I-evoked increase in renal plasma flow and a defect in IGF-I inotropic effect, which depends on a NO mechanism.

Moreover, our evidence is in accord with several experimental findings indicating that impairment of the endothelial NO dysfunction is related only to stimulated NO release, because L-NAME induced a similar vasoconstriction in both rat strains. This observation is supported by recent studies describing a preserved tonic and an altered phasic NO release in hypertensive rats.

Interestingly, IGF-I interacts with an endothelial NO mechanism at both high and low doses, which are able to evoke a net vasodilation or only an attenuation of vasoconstriction, respectively. This dose-dependent vascular response to IGF-I is likely related to different activation thresholds of the endothelial NO mechanisms. In other words, although high levels of IGF-I clearly activate the production of NO, lower levels of the hormone are only able to sensitize the NO production induced by other heterologous stimuli. Regarding this issue, we have also identified that the sensitization of heterologous NO-mediated vasodilations is not noticeable with any agonist-evoked vasorelaxation. Actually, IGF-I selectively sensitizes α2-adrenergic-evoked vasodilation, and our evidence is in accord with several experimental findings indicating that insulin, a factor that has a great similarity to IGF-I, can modify responsiveness to agents that operate via G protein, the main mediator of the α2-adrenergic receptor signaling pathway. It has also been demonstrated that endothelial α2-adrenergic-evoked relaxation participates in the whole vascular response evoked by norepinephrine, counterbalancing its opposite vasoconstrictive effects. Therefore, in light of our data, the attenuation of norepinephrine vasoconstriction exerted by low levels of IGF-I may be dependent on the sensitizing effect of the hormone on α2-adrenergic-evoked NO vasorelaxation. This hypothesis is sustained by our further observation that IGF-I sensitization of α2-adrenergic vasorelaxation is defective in SHR.

Because IGF-I has important growth-promoting features that play a major role in vascular remodeling during the development of and as a consequence of chronic pressure overload, we can speculate that the concomitant lack of vasorelaxant properties of IGF-I may contribute to the onset of higher blood pressure levels.

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