Insulin-Like Growth Factor I Diminishes in Vivo and in Vitro Vascular Contractility: Role of Vascular Nitric Oxide*

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ABSTRACT

Although most insulin-like growth factor I (IGF-I) in the circulation is generated by the liver, the hormone is also produced locally by the vasculature, suggesting its potential importance in regulation of regional blood flow. Accordingly, we studied the effects of in vivo exposure to IGF-I (5.1 nmol, iv) as well as in vitro incubation (100 nM) on endothelium-intact rat tail artery contractile responses to KCl and norepinephrine (NE). Systemic administration of IGF-I resulted in transient lowering of blood pressure, with maximal reduction occurring at 15 min and a return to baseline by 60 min. Maximal contractility of rings removed from animals 90 min after a bolus injection of IGF-I, when blood pressure had returned to normal, was significantly reduced for both KCl (58%) and NE (51%) without a change in sensitivity. Similar data were obtained when rings from untreated animals were preincubated in vitro for 90 min; maximal contractility in response to KCl was decreased by 31% and that to NE by 22%.

L-Nitroarginine methyl ester, an inhibitor of nitric oxide (NO) production, administered in vivo before IGF-I or added to the bath buffer reversed the attenuation. The nearly identical in vivo and in vitro results suggest that the observed diminution in contractility is a direct effect of IGF-I on the vasculature, probably mediated in large part by the release of NO. This idea is supported by our observation that IGF-I stimulates NO production in intact vessels. Further, the latency required indicates that rather complex mechanisms involving actions common to both receptor- and nonreceptor-mediated events are initiated by IGF-I and/or NO. (Endocrinology 137: 1798-1803, 1996)

INSULIN LIKE growth factor I (IGF-I) has long been associated with growth and differentiation. However, it is now generally accepted that it also exerts many metabolic effects similar to those of insulin, including glucose uptake, glycogen synthesis, and amino acid transport (1, 2). There is preliminary evidence that, like insulin, IGF-I may contribute to the regulation of vascular tone (3–6). Several studies suggest that the vascular actions of insulin vary in different vascular beds: inhibition of contraction in femoral, aortic, and tail vasculature and enhancement of it in mesenteric arteries (7–9). Regional differences in vascular responses to IGF-I may exist as well (9). Infusions of recombinant human IGF-I into both humans and rats increase renal blood flow and decrease vascular resistance in that organ (10, 11). Unlike insulin, however, IGF-I is produced locally (12) and, therefore, may potentially be a more important regulator of regional blood flow.

The endothelium-derived relaxing factor, nitric oxide (NO), is believed to be an important mediator of both insulin- and IGF-I-induced vascular relaxation (13–17), but other mechanisms have also been implicated (18–20). Systemic infusions of either insulin or IGF-I can elicit vasodilation through metabolic processes (21) and activate a number of counterregulatory mechanisms (22, 23), making interpretation difficult. In vitro, isolated vessels require preexposure to insulin or IGF-I (5, 9, 24), suggesting that complex mechanisms are involved in the vascular actions of these hormones. Accordingly, we used a dual ex vivo/in vitro approach to examine the role of NO in mediating the effects of IGF-I on the rat tail artery, a prototypical resistance vessel.

Materials and Methods

Animals

Male Wistar rats, 250–300 g, from Harlan (Indianapolis, IN) were housed two to a cage and given food and water ad libitum. Before each experiment, animals were fasted for 18–24 h and anesthetized with a mixture of urethane (1 g/kg) and α-chloralose (70 mg/kg). Animals were fasted to preclude variations in plasma glucose and postabsorptive state related to ad libitum feeding. Previous experience in our laboratory indicates that vascular reactivity is not significantly different between the fed and fasted states.

Intravenous injection of IGF-I

Rats were tracheotomized and placed on a heating pad to maintain body temperature. Catheters for infusion and blood pressure monitoring were inserted into the femoral artery and vein, and cardiovascular responses were measured using a Micro 5000 signal processing system (Modular Instruments, Melvern, PA) and the Bio Windows software program (Modular Instruments). After establishment of stable readings, IGF-I (human recombinant IGF-I; generous gift from Genentech, South San Francisco, CA; 5.1 nmol/animal) was injected into the femoral vein.
over a 1.5-min period. Initially, the dose of IGF-I selected was equiparal to that found to be effective for insulin administration, or 10.2 nmol/animal (25). However, this dose resulted in a profound irreversible hypotension in the animals, and it was reduced. The IGF-I obtained from Genentech in a citrate buffer, was further diluted in normal saline. Control animals for the in vivo studies were anesthetized, fully instrumented, and received an equal volume of saline. However, because of equipment and experimental design considerations, controls for the ex vivo experiments were anesthetized at the same time as the experimental animals, but not catheterized.

At that time, the animal was killed by pneumothorax, and the artery was removed for contractility studies. The following time points were selected for statistical analyses: 0, 5, 15, 30, 45, and 60 min, although mean arterial pressures and heart rate were monitored for 90 min (25). Before the start of the experiment and 30 min after injection, a 0.3-ml blood sample was collected for glucose measurement (Sigma Chemical Co., St. Louis, MO). The animals were replaced with an equal volume of heparinized warmed saline. All animals were treated in accordance with institutional guidelines.

**Vascular contractility**

After death, the rat tail artery was dissected free and placed in ice-chilled buffer containing 130 mol/L NaCl, 15 mol/L NaHCO₃, 4.7 mol/L KCl, 1.2 mol/L KH₂PO₄, 1.6 mol/L CaCl₂, 1.2 mol/L MgSO₄, 0.03 mol/L EDTA, and 5.6 mol/L glucose. Three millimeter rings were suspended from isometric force transducers (Gould Instruments, Cleveland, OH) in muscle baths containing the same buffer at around 37°C and aerated with 95% O₂-5% CO₂ to maintain pH. All rings were stretched to 1 g of tension and allowed to stabilize for 30 min before the addition of agonists (24). In experiments in which rings were preincubated in vitro, IGF-I (10 and 100 nm) was added 30, 60, or 90 min before agonist addition (1.0-120 nm KCl or 1 nm to 10 μm norepinephrine (NE)). To evaluate the mode of nitric oxide, 1-nitroarginine methyl ester (L-NAME; 100 μM) was added 5 min before IGF-I. Acetyl choline (10 μM) was used to test endothelial integrity. All chemicals and drugs were purchased from Sigma.

**Vascular NO production**

Rat tail arteries did not provide enough tissue mass for the following studies, so aorta was used instead. The vessels were removed from Sprague-Dawley rats and carefully cleaned of fat to maintain the endothelium. Seven vessels were then incubated in an organ chamber in the buffer used for contractility under constant aeration with 95% O₂-5% CO₂ to maintain pH. All rings were stretched from isometric force transducers (Gould Instruments, Cleveland, OH) in muscle baths containing the same buffer at around 37°C and aerated with 95% O₂-5% CO₂ to maintain pH. All rings were stretched to 1 g of tension and allowed to stabilize for 30 min before the addition of agonists. After in vitro exposure (Fig. 3). Maximal contractility was significantly decreased (1316 ± 163 vs. 1986 ± 94 mg in control rings for NE; 1556 ± 77 vs. 1775 ± 67 mg in controls for NE) without a change in sensitivity (ED₅₀ for NE, 10.9 ± 3.1 vs. 5.2 ± 1.8 μM in controls, for KCl, 10.2 ± 1.6 vs. 8.4 ± 0.8 mm in control rings). Preincubation with 10 mm L-NAME for the same time period also tended to decrease contractility, but the effect was not significant (19% for NE and 26% for KCl compared to controls), but exposure for shorter time periods (60 and 30 min) had no effect (data not shown). These results coupled with those of the ex vivo experiments indicate that IGF-I has a direct impact on the vasculature that persists after in vivo exposure (25).

**Statistical calculations**

ED₅₀, values for dose-response curves were calculated with the All Fit program, and differences were determined by Student's t test. Two-way (with Fisher's protected least significant difference) and repeated measures ANOVAs were used as warranted to compare contractility and blood pressure changes between different treatment groups. Values in the text are given as the average ± SEM, and P < 0.05 is considered significant.

**Results**

**In vivo**

Systemic infusion of 5.1 nmol IGF-I as a bolus significantly, but transiently, decreased blood pressure in male Wistar rats. As shown in Fig. 1, the effect was maximal at 15 min (14.1 ± 3.0%; P < 0.001), but blood pressure was normalized by 60 min. Plasma glucose was significantly reduced when measured 30 min after IGF-I injection (54.9 ± 9.3 vs. 98.2 ± 7.3 mg/dl at baseline; P < 0.01, by paired t analysis in 11 ani-
IGF-I DIMINISHES VASCULAR CONTRACTILITY

Fig. 2. In vitro contractility in rat tail artery rings 90 min after an iv bolus of IGF-I (5.1 nmol; as described in Materials and Methods). Maximal tension achieved was significantly lower in response to both KCl (top panel) and NE (bottom panel; n = 5 paired experiments each). P = 0.0002 and 0.0001, respectively, by two-way ANOVA.

± 232 vs. 920 ± 270 mg in control tail artery segments with an ED$_{50}$ of 4.1 ± 3.4 vs. 5.3 ± 4.5 uM for NE in controls). In vitro, preincubation with L-NAME (100 nM) for 5 min before the addition of IGF-I (100 nM for 90 min) completely reversed the effect of IGF-I, enhancing maximal contractility to NE by 25% and to KCl by 26% over those in IGF-I-treated rings (Fig. 4). The maximal tension achieved in response to NE in the original experiments was 1981 ± 77 mg in control rings and 1556 ± 180 in IGF-I treated rings; with L NAME, tension was 1864 ± 191 compared to 1496 ± 188 mg in rings exposed to IGF-I only, indicating that a complete reversal of the IGF-I effect was achieved with prior exposure to L-NAME. Again, no changes in ED$_{50}$ could be detected.

Vascular NO generation

As shown in Fig. 5, 90-min incubation (when diminished vascular contractility was first observed) of intact vessels resulted in the release of NO into the medium; this was significantly enhanced in the presence of IGF-I (100 nM; P < 0.05). As the technique used to measure NO production, the Greiss reaction, measures accumulated NO$_2$/NO$_3$, the significant elevation at 90 min reflects production over the 90-min exposure to IGF-I. Similar results were obtained when endothelium-denuded aortic strips or vascular smooth muscle cells (VSMC) in culture were incubated with 100 nM IGF-I for 90 min (data not shown), suggesting that endothelium was not the sole source of IGF-I-stimulated NO release.
Discussion

Our results indicate that IGF-I attenuates both receptor-mediated and voltage-induced vascular tone predominantly through direct actions on the vessel wall. We chose the rat tail artery, a highly innervated and reactive vessel, rather than the aorta for the contractility studies. Our data demonstrate that IGF-I reduces contractility in the tail artery, much as has been reported in the aorta (9) and renal artery (10, 11), but unlike that in the mesenteric artery (9). This similarity in aortic and tail artery responses to IGF-I led us to use aorta for the NO release experiments; rat tail artery rings did not provide enough material for determination of IGF-I-induced NO production. IGF-I appears to function like insulin in human skeletal muscle blood vessels (6, 14-17). That these two hormones have comparable effects in a reactive vessel such as the rat tail artery as well as in other vascular beds is not surprising because they share similar receptors and mechanisms of action (26, 27).

Local mechanisms by which IGF-I may decrease vascular reactivity include activation of Na',K'-adenosine triphosphatase (Na',K'-ATPase), changes in intracellular Ca', interactions with α- and β-adrenergic receptors, and release of an endothelium-dependent relaxing factor, among others (28). Although IGF-I has been shown to enhance aortic Na',K'-ATPase activity (29), a possible mechanism for its vascular effects, most reports are limited to the actions of insulin on the vessel wall. Previous studies in our laboratory and those of others have demonstrated a direct effect of insulin on VSMC (4, 5, 30, 31). In particular, insulin can inhibit agonist- and voltage-induced VSMC intracellular calcium [Ca'] transients, thus leading to decreased vascular contraction (4, 30, 31). Another possible mechanism by which insulin may attenuate [Ca'] is by selective antagonism of α,-receptors, as has been reported by Lembo et al. (19). Alternatively, insulin has been shown to both decrease β-adrenergic receptor binding (32) and down-regulate β,-receptor activity via phosphorylation (33). More directly, norepinephrine release into mesenteric perifusates is lowered in the presence of insulin (34) via a presynaptic inhibitory mechanism. Any or all of these actions could be invoked for IGF-I, but most do not adequately account for the lag time required in the isolated vessel in vitro or for the fact that contractile responses to both NE and KCl are impaired. Indeed, the significant decrease in plasma glucose seen after IGF-I infusion indicates that a number of counterregulatory responses, some adrenergic, are called into play to restore glucose homeostasis and normalize blood pressure. However, the ex vivo reduction in contractility persisted for at least 2 h after blood pressure had returned to baseline values in the intact animal. These results suggest that a persistent modification of the intracellular milieu and/or the contractile apparatus itself has been initiated by IGF-I, alone or through the release of a mediator.

That much of the IGF-I attenuation of vascular contractility can be reversed by L-NAME supports data from other laboratories indicating that both insulin and IGF-I mediate this action through the release of NO (14, 15, 21). Both the constitutive, Ca'/calmodulin-dependent, NO synthase (cNOS) and the inducible, Ca'/calmodulin-independent, form of...
the enzyme (iNOS), are found in the vessel wall (35). cNOS may be activated by serine phosphorylation, followed by its 
translocation from the membrane to the cytosol (36). This is 
an unlikely mode of control for IGF-I unless an intermediate 
kinase can be implicated. In addition, IGF-I can stimulate NO 
release from endothelial cells without increasing [Ca\(^{2+}\)] (13), 
suggesting that control of the enzyme is at least multifactor-
ial. iNOS, on the other hand, is believed to be totally tran-
scriptionally regulated, generally by inflammatory cytokines 
(37). This process is inhibited by tyrosine kinase inhibitors, 
such as genistein and tyrphostin (38), making this enzyme 
amenable to control by IGF-I, whose receptor itself is a ty-
rosine kinase. In one report, concomitant exposure of vas-
cular smooth muscle cells to cytokines and IGF-I reverses the 
cytokine induction of iNOS, but only one time point was 
examined (39). The NO generated by either enzyme may 
relax muscular contraction via activation of guanylate cy-
clase. This, in turn, activates the sarcoplasmic reticulum 
Ca\(^{2+}\)-ATPase and sequestration of Ca\(^{2+}\) (40). NO may also 
act in a cGMP-dependent manner to hyperpolarize the cell 
via an increase in the opening probability of the Ca\(^{2+}\)-sen-
sitive K\(^+\) channel (41). Both mechanisms may account for 
part of the immediate relaxation seen after agonist-induced 
cNOS activation, but they may also contribute to the pro-
found and long lasting hypotension that occurs after cyto-
kine stimulation of iNOS.

The endothelium possesses IGF-I receptors (42) and en-
dothelial cells (13), and intact vessels release NO in response 
to IGF-I, suggesting that both paracrine and autocrine effects 
of IGF-I may be mediated in part through NO production. 
Nevertheless, in our hands, IGF-I added directly to precon-
tracted, endothelium-intact rings generates only a minor and 
transient relaxation (data not shown), and long preincubation 
times are required to observe substantial vasorelaxation, 
similar to earlier observations for insulin (9, 24). This delayed 
relaxing effect of IGF-I differs from the in vivo situation, 
where relaxation is immediate, as would be expected from 
endothelial NO release. Vessels in vitro are not exposed to 
tonic NO generated by shear stress and are more tonically 
contracted than those in vivo. However, unlike IGF-I, ace-
tylycholine produces an immediate and profound relaxation 
when added to our cell preparations. Thus, the possibility 
exists that not only the cNOS found in endothelial cells, but 
also the inducible synthase of both endothelial and vascular 
smooth muscle cells could be activated by IGF-I, which is 
constitutively produced by VSMC (12, 43, 44). Our new ob-
ervation that IGF stimulates a vascular increase in NO after 
90 min is consistent with such a possibility. Ignarro et al. (45) 
have shown that NO is continuously released by both en-
dothelial and vascular smooth muscle cells in the pulmonary 
circulation and that the release may be regulated by several 
different mechanisms. In addition, Marczin et al. (46) re-
ported that inhibition of tyrosine kinase activity (an early 
postreceptor event in IGF-I and insulin action) suppresses 
NO production by VSMC. The fact that dexamethasone (an 
inhibitor of iNOS induction) suppresses insulin-mediated 
vasodilation (16) suggests that iNOS stimulation is a possible 
mechanism for the delayed attenuation of contractility seen 
with both insulin and IGF-I. Our results do not allow us to 
discern whether the production of NO is via stimulation of 
cNOS or iNOS in the endothelium, the VSME, or both, al-
though the time course required for IGF-I to exert its vaso-
dilatory properties is consistent with stimulation of both. 
Alternatively, either IGF-I and/or endothelial cell-induced NO 
may initiate transcription of a protein(s) involved in the 
regulation of vascular contraction (47). Again, we cannot 
discriminate between these two possibilities. However, we 
can conclude that whatever the mechanism, it must be com-
mon to both receptor- and nonreceptor-mediated signaling 
events, because NE- and KCl-induced contractions were sim-
ilarly attenuated. As such, it may be similar to the direct effect 
postulated by Hollenberg for epidermal growth factor, an-
other hormone acting via a tyrosine kinase, which produced 
the same reduced response to both agonists (48).

In conclusion, the results of this investigation demonstrate 
that IGF-I has a direct impact on the vasculature and atten-
uates contractility in the rat tail artery, an effect related to NO 
production. As IGF-I is not only secreted by VSMC, but also 
stimulates vascular NO production, this hormone may play 
a significant paracrine/autocrine role in the regulation of 
local blood flow. However, the relatively long lag time re-
quired for the actions of IGF-I on the vasculature suggests 
that relatively complex mechanisms are involved.

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