Mechanism of coronary vasodilation to insulin and insulin-like growth factor I is dependent on vessel size

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Oltman, Christine L., Neal L. Kane, David D. Gutterman, Robert S. Bar, and Kevin C. Dellsperger. Mechanism of coronary vasodilation to insulin and insulin-like growth factor I is dependent on vessel size. Am J Physiol Endocrinol Metab 279: E176–E181, 2000.—Insulin and insulin-like growth factor I (IGF-I) influence numerous metabolic and mitogenic processes; these hormones also have vasoactive properties. This study examined mechanisms involved in insulin- and IGF-I-induced dilation in canine conduit and microvascular coronary segments. Tension of coronary artery segments was measured after constriction with PGF2α. Internal diameter of coronary microvessels (resting diameter 112.6 ± 10.1 μm) was measured after endothelin constriction. Vessels were incubated in control (Krebs) solution; dogs; potassium channels diabetes mellitus; coronary circulation; coronary microcirculation; IGF-I; nitric oxide synthase (NOS) and cyclooxygenase inhibition and was blocked with KCl constriction. Coronary microvascular relaxation to insulin and IGF-I was not altered by L-NA, indomethacin, tetraethylammonium chloride, glibenclamide, charybdotoxin, and apamin; however, tetrabutyrammonium chloride attenuated the response. In conclusion, insulin and IGF-I cause vasodilation in canine coronary conduit arteries and microvessels. In conduit vessels, NOS/cyclooxygenase pathways are involved in the vasodilation. In microvessels, relaxation to insulin and IGF-I is not mediated by NOS/cyclooxygenase pathways but rather through K+-dependent mechanisms.

No previous study has examined the vascular effects of insulin and IGF-I on conduit and microvascular arteries of the heart, an organ that is impacted upon by multiple processes in diabetes. In this report, we tested the hypothesis that insulin and IGF-I have different responses and mechanisms of action in coronary conduit vs. microvascular vessels. We further explored potential mechanisms responsible for the hormonally induced vasodilation.

METHODS

All protocols were approved by the University of Iowa Animal Care and Use Committee and the Veterans Affairs Research Committee and conform to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication no. 85–23, revised 1985).

Animals and tissue. Sixty-five adult mongrel dogs of either sex (5–8 kg) were killed with an overdose of pentothal sodium (50 mg/kg). Hearts were removed and immediately placed in cold (4°C), oxygenated (20% O2-5% CO2-75% N2) Krebs bicarbonate buffer solution (see Solutions and drugs). Coronary conduit arteries and ventricular microvessels were isolated and cleaned of fat and connective tissue. Isolated vascular rings. Canine coronary arteries were studied using a standard isometric ring technique (13, 14). Segments of the right coronary, left circumflex, or left anterior descending coronary arteries (1–2 mm in diameter) were dissected and cut into 5-mm ring segments. In some ring segments, endothelium was removed by pulling silk sutures

INSULIN AND insulin-like growth factor I (IGF-I) are structurally related peptides capable of stimulating a variety of metabolic and mitogenic processes. Receptors for both hormones are heterodimeric transmembrane tyrosine kinase receptors that, when stimulated, activate multiple intracellular pathways, resulting in altered metabolic and growth regulatory processes (9). IGF-I has been proposed as a novel therapy for patients with type 1 diabetes mellitus and for severely insulin-resistant patients with type 2 diabetes. Previous studies examining the vascular effects of insulin and IGF-I have yielded conflicting results. Some studies have shown that insulin or IGF-I enhanced tone or caused constriction (18, 24). Other studies have shown vasodilation that is either nitric oxide synthase (NOS) dependent (3, 6, 10, 17, 19, 22–24) or nitric oxide (NO) independent (11). The disparate effects may be due to differences in species or vascular beds studied. For example, Juncos and Ito (7) showed that insulin caused dilation of efferent but not afferent arterioles of the rabbit kidney, demonstrating regional variation, even within an organ.

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through the vessel. Vascular rings were mounted on two stainless steel wire stirrups passed through the vessel lumen. One stirrup was attached to a force transducer (Grass FT03), and the other was attached to a micrometer microdrive to allow the vessel to be stretched by small increments. Each vessel apparatus was placed in a 10-ml jacketed organ bath containing Krebs buffer equilibrated at 37°C and aerated with 20% O₂-5% CO₂-75% N₂. Isometric contractions and relaxations were measured on a physiological recorder. Coronary rings were individually stretched to the maximum of the length-developed tension relationship by repeated test exposures to 75 mM KCl at increasing vessel tension.

The coronary artery segments were allowed to stabilize 30 min before concentration-response curves in either control solution (normal Krebs) or Krebs solution with N^3-nitro-L-arginine (L-NA, 100 μM) and indomethacin (Indo, 10 μM). PGF2α (6–10 μM) or KCl (30–40 mM) was used to constrict the vessels to 30–50% of their resting tension. After steady-state tension was achieved, a concentration-response curve to insulin (0.1–100 ng/ml) or IGF-I (0.1–100 ng/ml) was performed. A single dose of sodium nitroprusside (SNP, 10^-4 M) was then administered to establish maximal smooth muscle vasodilation. Acceptable coronary artery ring experiments met the following criteria: 1) developed tension to PGF2α >1 g; 2) 80–150% dilation to SNP; and 3) <30% dilation to bradykinin (10^-6 M) in denuded vessels.

**Isolated microvessels.** A standard in vitro pressurized arteriole preparation was used to study coronary microvessels (13, 14). Ventricular microvessels (75–175 μm intraluminal diameter and ~1 mm in length) were carefully removed from the myocardium and cleaned with the aid of a dissecting microscope (Olympus SZ-6045 stereo zoom microscope). Each end of the microvessel was cannulated with a glass micropipette and secured with 10–0 ophthalmic suture. The cannulated pipettes were attached to a hydrostatic pressure reservoir (20 mmHg) under conditions of no flow. The organ chamber was placed on the stage of an inverted microscope (Olympus CK40). Attached to the microscope were a video camera, a video monitor, and a calibrated video caliper (Boeckler Video encoder). The organ chamber was connected to a rotary pump that continuously circulated oxygenated Krebs buffer warmed to 37°C. An image of the microvessel was displayed on the video monitor, and intraluminal diameters were measured by manually adjusting the video micrometer. The resolution of the system allowed measurement of very small (1–2 μm) changes in vessel diameter.

Microvessels were allowed to equilibrate for 30 min at a distending pressure of 20 mmHg. KCl (50 mM) was added to the bath to test constrictor capacity. Microvessels were incubated for 30 min in Krebs buffer alone (control) or in the presence of one or more of the following inhibitors: Indo (10 μM), L-NA (100 μM), glibenclamide (1 μM), tetraethylammonium chloride (TEA, 1 mM or 10 mM), tetrabutylammonium chloride (TBA, 1 mM), 4-aminopyridine (4-AP, 0.5 mM), or combinations of L-NA and Indo, glibenclamide and TEA, or charbydotoxin (50 nM) and apamin (500 μM). To mimic the effects of euglycemic and hyperglycemic conditions, responses to insulin and IGF-I were studied using low (5.5 mM) and high (22 mM) glucose concentrations. The standard solution used in this study contained 11 mM glucose. KCl constriction was used in some vessels to examine the role of K⁺ channels on insulin- and IGF-I-induced dilation. Both hypertonic (addition of KCl to the bath) and isotonic (equimolar reduction of NaCl) solutions were used. Endothelin-1 (0.40–8.0 nM) was used to constrict microvessels to 30–60% of their resting diameter. Cumulative concentration-response relationships were evaluated for insulin or IGF-I (0.1–100 ng/ml) by adding the drug directly to the organ bath. A single dose of SNP (10^-6 M) or papaverine (10^-4 M) was given at the end of the experiment to determine maximal dilation. The following criteria were required for an acceptable microvessel experiment: 1) no obvious leaks; 2) constriction of >50% to 50 mM KCl and >30% to endothelin; and 3) dilation of >80% to 10^-4 M SNP or 10^-4 M papaverine.

**Solutions and drugs.** Krebs solution contained (in mM): 120.0 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 23.0 NaHCO₃, 1.2 K₂HPO₄, 11 glucose, and 0.025 EDTA. Solutions were aerated with 20% O₂-5% CO₂-75% N₂ and were warmed to 37°C with pH maintained at 7.4. Insulin was obtained from Eli Lilly (Indianapolis, IN) and was diluted in 0.01 normal HCl. IGF-I was obtained from Intergen (Purchase, NY) and was diluted in 0.05 M acetic acid. Insulin and IGF-I were stored at −20°C until ready to use. Endothelin-1 was purchased from Peninsula Laboratories (San Carlos, CA). All other inhibitors and vasoactive agents were purchased from Sigma Chemical (St. Louis, MO). All solutions and vasoactive agents were prepared fresh on the day of the experiment.

**Statistical analysis.** Results are expressed as percent dilation, with 100% representing the difference from the constricted value with endothelin, PGF2α, or KCl to the resting value (tension or diameter). All concentration-response curves were evaluated for changes in maximal responses and differences at each dose using ANOVA with repeated measures and the Fisher least-significant difference correction for multiple comparisons. Comparisons of percent vasodilation under different treatment conditions were performed. Significance of differences among mean values of resting conditions, maximal effect, and EC₅₀ values was assessed with a one-way ANOVA. Data are expressed as means ± SE; n indicates the number of animals. Differences with P < 0.05 were considered significant.

**RESULTS**

**Canine coronary conduit artery responses.** Resting tension of conduit artery rings was 2.8 ± 0.1 and 2.7 ± 0.1 g in insulin and IGF-I control groups, respectively, and was not different among any of the treatment groups [P = not significant (NS)]. Developed tension to PGF2α was similar among groups [2.0 ± 0.2 and 2.6 ± 0.6 g in insulin and IGF-I control groups, respectively (P = NS)]. In canine coronary arteries constricted with PGF2α, both insulin and IGF-I produced relaxation (Fig. 1). The highest concentration of IGF-I (100 ng/ml) induced 68 ± 8% relaxation and was greater than the relaxation induced by 100 ng/ml insulin (33 ± 4%). Insulin and IGF-I did not produce relaxation in vessels that had endothelial cells removed (−1.4 ± 8.8% dilation for insulin and 11.3 ± 6.1% dilation for IGF-I at 100 ng/ml, n = 4). Cyclooxygenase (Indo, 10 μM) and NOS (L-NA, 100 μM) inhibition blocked the insulin-induced dilation and modestly attenuated (46%) the IGF-I-induced dilation (Fig. 1). When coronary rings were constricted with KCl (30–40 mM), relaxation to insulin and IGF-I was completely abolished (Fig. 1).

**Coronary microvascular responses.** Baseline diameter was 107 ± 17 and 102 ± 16 μm (P = NS) for microvessels in the control groups for insulin and IGF-I, respectively. Baseline diameters were not different between the control and any of the treatment groups. Microvessels were constricted with endothelin (0.4–8.0 nM) to 43 ± 4 and 49 ± 5% resting diameter.

**INSULIN- AND IGF-I-INDUCED VASODILATION**
in the control insulin and IGF-I groups, respectively. Insulin and IGF-I produced concentration-related relaxation in canine coronary microvessels. The vasodilator responses to insulin and IGF-I were similar, with maximal relaxation of 69 ± 6% for insulin and 69 ± 7% for IGF-I (Fig. 2). Inhibition of NOS, cyclooxygenase, or both pathways did not alter insulin- or IGF-I-induced vasodilation in coronary microvessels (Fig. 3). Thus NOS/cyclooxygenase pathways appear not to be necessary in insulin/IGF-I-induced vasodilation in coronary microvessels, in contrast to their role in the coronary conduit arteries.

Constriction with hypertonic KCl solutions (39 ± 4 mM for insulin and 40 ± 5 mM for IGF-I) abolished dilation to insulin and IGF-I, except at the highest dose of 100 ng/ml (Fig. 4). This concentration of KCl produced similar amounts of constriction as endothelin and should prevent dilation due to K⁺ efflux from smooth muscle cells. Similar responses were found using isotonic (NaCl replacement) solutions (data not shown). These results suggest that K⁺ efflux mechanisms play a role in insulin and IGF-I-induced vasodilation in coronary microvessels.

To determine whether specific K⁺ channels are involved in insulin- and IGF-I-induced dilation, specific inhibitors of K⁺ channels known to be present in the coronary circulation were tested. TBA (1 mM), a nonspecific calcium-sensitive K⁺ channel inhibitor, significantly attenuated insulin- and IGF-I-induced dilation (Fig. 5) at 10–100 ng/ml for insulin and 30–100 ng/ml for IGF-I. Glibenclamide (1 μM), an ATP-sensitive K⁺ channel inhibitor, TEA (1 mM), a large-conductance Ca²⁺-activated K⁺ (KCa) channel inhibitor, and the combination of glibenclamide and TEA did not alter insulin- and IGF-I-induced dilation (Fig. 6). Tenfold higher concentrations of TEA (10 mM) also did not alter relaxation to insulin and IGF-I (response at 100 ng/ml: insulin: 65 ± 5, n = 4; IGF-I: 65 ± 11, n = 5). These data suggest that K⁺ channels other than ATP-sensitive and large-conductance KCa channel inhibitors, respectively) together did not alter dilation to insulin or IGF-I (Table 1).

Insulin- and IGF-I-induced dilation was also evaluated in “euglycemic” (5.5 mM, n = 5) and “hyperglycemic” (11 and 22 mM, n = 5) solutions. Vessels were in the modified glucose solutions for at least 1 h before the insulin or IGF-I curves were performed. Neither decreasing nor increasing the glucose concentration altered the vasodilation to insulin or IGF-I (data not shown).

**DISCUSSION**

In this study, insulin and IGF-I produced vasodilation in both canine coronary conduit arteries and microvessels. However, the mechanisms responsible for vasodilation differed in the two coronary vascular beds. In coronary conduit arteries, insulin produced less relaxation than IGF-I. Also, in conduit arteries, vasodilation induced by both hormones was endothelium dependent, was attenuated by NOS and cyclooxygenase inhibition, and was completely blocked when KCl was used to con-
strict the vessels. In canine coronary microvessels, relaxation to insulin was similar to IGF-I-induced relaxation. However, in contrast to conduit arteries, this relaxation was not mediated by NO, cyclooxygenase, ATP-dependent K⁺ channels, or small or large KCa channels; rather, hyperpolarization, via some other K⁺ channel(s), appeared to be involved in the vasodilation.

Previous studies that focused on the vasoactive properties of insulin and IGF-I yielded conflicting results (7, 10, 11, 17, 18, 22, 24). Differences among these studies include the vascular bed studied, vessel size examined, and methodological diversity. Few studies examined the effects of insulin and IGF-I in the coronary circulation. Recently, Hasdai et al. (5) showed that insulin and IGF-I produced 28 and 25% relaxation, respectively, of endothelin-constricted porcine coronary conduit arteries. This relaxation was endothelium independent and involved smooth muscle cell K⁺ channels. We also saw modest relaxation to insulin (32%) in canine coronary arteries. However, in our study, IGF-I produced a greater amount of vasodilation than insulin (66 vs. 32%). The current study also found that insulin- and IGF-I-induced relaxation was endothelial dependent in canine conduit arteries. Hasdai et al. did not investigate microvascular responses to insulin and IGF-I in the porcine coronary circulation.

Selective differences in insulin- and IGF-I-induced vasodilation between conduit coronary arteries and coronary microvessels were present. In this study, L-NA and Indo attenuated the dilation to insulin and IGF-I in conduit arteries; however, the same combination of inhibitors did not alter microvascular dilation. McKay and Hester (10) have shown that insulin-induced vasodilation in hamster cremaster muscle is NO dependent in second-order but not in third- or fourth-order arterioles. Kersten el al. (8) and Nagao et al. (12) reported that smaller blood vessels exhibited enhanced potency to aprikalim, an agonist that produces vasorelaxation through activation of ATP-sensitive K⁺ channels, suggesting that small arteries are more sensitive to the actions of hyperpolarizing vasodilators than are large arteries.

Our results also show that K⁺ channels mediate some of the insulin- and IGF-I-induced vasodilation in both coronary conduit arteries and microvessels. High
extracellular K⁺ concentrations blocked insulin- and IGF-I-induced dilation in both coronary conduit arter-
ies and microvessels. In coronary microvessels, the nonspecific K⁺ channel inhibitor TBA attenuated the
response to insulin and IGF-I. However, when studies were performed to block a specific class of K⁺ channels,
using glibenclamide (1 μM), an ATP-sensitive K⁺ channel inhibitor, or TEA (1 mM), a K<sub>Ca</sub> channel in-
hibitor, normal vasodilation was observed to insulin and IGF-I. Comparable relaxation was also observed in
the presence of the combination of glibenclamide and TEA or at a higher concentration of TEA (10 mM).

There are several possible explanations for the K⁺ channel findings of our study. The inhibitors chosen in
this study may not completely inhibit their respective classes of K⁺ channels (4, 16). A distinct type of K⁺
channel may be involved in insulin- and IGF-I-induced dilation that is not blocked by the inhibitors used in
our study. Insulin and IGF-I may produce vasodilation through redundant pathways or diverging signal cas-
cades, which in turn affect K⁺ channel activity in different ways.

**Limitations of the study.** Our study did not address the reactivity of coronary vessels from diabetic animals
in response to insulin and IGF-I. Previous investigations have demonstrated that some vascular dysfunc-
tion associated with diabetes can be mimicked using hyperglycemic solutions (15, 20, 21). However, in this
study, neither high nor physiological glucose concentrations altered insulin- or IGF-I-induced vasodilation
in coronary microvessels. The use of euglycemic and hyperglycemic solutions to assess the potential effect of
varying levels of glycemia on insulin and IGF-I may mimic the hyperglycemia observed in diabetes; how-
ever, it does not produce the potential adverse effects on the vasculature that can result from chronic hyper-
glycemia. Studies using vessels from diabetic animals could produce different results.

Systemic circulating levels of insulin are ~0.2 ng/ml in the fasting state, which increase to 2 ng/ml after a
glucose load. In the portal circulation, the concentra-
tion of insulin can be 10 ng/ml or higher. In patients
with insulin resistance, plasma levels of insulin can be
>5 ng/ml. IGF-I is present in the circulation in micro-

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**Fig. 5.** Effect of tetrabutylammonium chloride
(TBA; 1 mM) on insulin- and IGF-I-induced va-
sodilation in canine coronary microvessels. When
microvessels were incubated for 30 min in the
presence of TBA, insulin- and IGF-I-induced va-
sodilation was significantly attenuated. This pro-
vides evidence that hyperpolarization via K⁺
channels plays a role in this dilation. *P ≤ 0.05
vs. control.

**Fig. 6.** Role of K⁺ channels in insulin- and IGF-
I-induced dilation of canine coronary microves-
sels. Insulin- and IGF-I-induced relaxation was
not altered when the microvessels were incu-
bated in the presence of glibenclamide, tetrathy-
lammonium chloride (TEA), or a combination of
both inhibitors.
In summary, insulin and IGF-I cause vasodilation in canine coronary conduit arteries and microvessels. In conduit vessels, endothelial-dependent NOS/cyclooxygenase pathways mediate insulin- and IGF-I-induced vasodilation. In coronary microvessels, relaxation to insulin and IGF-I is similar and is not mediated by NO/cyclooxygenase pathways, but rather by hyperpolarization through K+ channels.

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REFERENCES


Table 1. Effect of additional K+ channel inhibitors on 100 ng/ml insulin- or IGF-I-induced microvascular relaxation

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<th>Insulin</th>
<th>IGF-I</th>
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<tr>
<td>Control</td>
<td>69 ± 6(11)</td>
<td>69 ± 7(8)</td>
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<tr>
<td>4-AP</td>
<td>65 ± 10(3)</td>
<td>85 ± 10(3)</td>
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<tr>
<td>Charbdotoxin + apamin</td>
<td>76 ± 6(3)</td>
<td>92 ± 9(3)</td>
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Values are means ± SE; no. of observations in parentheses. IGF-I, insulin-like growth factor I; 4-AP, 4-aminopyridine. In the presence of 4-AP (200 μM) or the combination of charbdotoxin (50 nM) and apamin (0.5 μM) together, canine coronary microvessels were constricted with endothelin. Insulin or IGF-I produced concentration-dependent vasodilation. These inhibitors did not alter dilatation to insulin or IGF-I. P = not significant vs. control.