Insulin and IGF-I attenuate the coronary vasoconstrictor effects of endothelin-1 but not of sarafotoxin 6c

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Abstract

Objective: To examine the hypothesis that insulin and insulin-like growth factor I (IGF-I) attenuate endothelin-induced contraction of porcine coronary epicardial arteries in vitro. Background: Endothelin-induced coronary vasoconstriction is mediated by two types of receptors, A (ETA) and B (ETB), resulting in calcium influx. Both insulin and IGF-I attenuate endothelin-induced calcium influx into porcine coronary artery smooth muscle. Methods: Epicardial arteries harvested from juvenile pigs were contracted with cumulative concentrations of endothelin-1 (ETA- and ETB-receptor agonist; 10⁻¹⁰–10⁻⁶ M) or of sarafotoxin-6c (ETB-receptor agonist; 10⁻¹¹–10⁻⁷ M). In additional experiments, endothelin-1 or sarafotoxin-6c were added after incubation with 10⁻⁷ M regular insulin or IGF-I. These experiments were repeated in vessels without endothelium. Contraction for each vessel was calculated relative to the response to 60 mM KCl. Results: The maximal contractions to endothelin-1 in vessels with and without endothelium were 158±8 and 200±21%, respectively (p<0.05 at 10⁻⁷–10⁻⁶ M). Both insulin (at 10⁻⁷–10⁻⁶ M) and IGF-I (at 10⁻⁸–10⁻⁶ M) attenuated the contraction to endothelin-1 in vessels with intact endothelium, as well as in vessels without endothelium (at 10⁻⁷ and 10⁻⁶ M for insulin and 10⁻⁷⁻¹₀⁻⁶ M for IGF-I). The maximal contractions to sarafotoxin-6c in vessels with and without endothelium were 54±13 and 84±7%, respectively (p<0.05 at 10⁻⁷, 10⁻⁸, and 10⁻⁷ M). Insulin and IGF-I did not affect the response to sarafotoxin-6c in vessels with and without endothelium. Conclusion: Insulin and IGF-I attenuated ETA-receptor-mediated coronary contraction through an endothelium-independent mechanism. The IGF axis may serve as an endogenous modulator of endothelin-mediated vasoconstriction.

Keywords: Pig; Insulin; Insulin-like growth factor I; Endothelin-1; Sarafotoxin-6c; Endothelin receptors; Coronary artery

1. Introduction

Endothelin-1 (ET-1) is a 21 amino acid peptide, cleaved from a 39 amino acid precursor, big ET-1, through proteolytic processing [1,2]. ET-1 is a potent coronary vasoconstrictor at pathophysiologic concentrations [3]. The effects of ET on vascular tone are mediated by two major types of ET receptors, A (ETA) and B (ETB), each encoded by a different gene [4,5]. The two receptor subtypes have distinct tissue distributions and affinities to ET isoforms: ETA receptors are present on vascular smooth muscle cells, whereas ETB receptors are found on both vascular smooth muscle and endothelial cells [6]. The ETA receptor is selective for the ET-1 over the ET-3 isoform, while the ETB receptor has similar affinities for all ET isoforms [5]. ET-induced vasoconstriction is mediated by calcium influx [2].

Insulin-like growth factors I (IGF-I) and II are 7.5 kDa peptides; both are highly homologous in structure and function to proinsulin [7–9]. The peptides belonging to the IGF family interact with specific receptors designated as type I and II IGF receptors, as well as with the insulin receptor [7]. The type I IGF receptor binds IGF-I with high affinity and insulin with low affinity, whereas the insulin receptor binds IGF-I with a much lower affinity than insulin. Both insulin and IGF-I have diverse vasoactive properties, ranging from vasodilatation to vasoconstriction, depending on the species examined, as well as on the particular vascular bed [10–25].

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Prior studies have demonstrated an interaction between the IGF and ET pathways [26–33]. Endothelin production is stimulated by insulin and IGF-I [26–32], and vice-versa [33]. There are few data regarding the coronary vasoactive interactions between the two pathways. Because both insulin and IGF-I attenuate ET-1-induced calcium influx into porcine coronary artery smooth muscle [34], we postulated that they would also attenuate ET-1-induced contraction of porcine coronary epicardial arteries in vitro.

2. Methods

2.1. Animals

The study procedures and handling of animals were reviewed and approved by the Mayo Foundation Institutional Animal Care and Use Committee. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1985). Juvenile domestic crossbred pigs were euthanized with an intravenous overdose of pentobarbital sodium (intravenous 30 mg/kg; Sleepaway, Fort Dodge Laboratories, IA, USA). After euthanization, the hearts were harvested for in vitro analysis.

2.2. In vitro analysis of epicardial arteries

In vitro determination of epicardial reactivity was performed as previously described [35]. In brief, the hearts were placed into cold modified Krebs–Ringer bicarbonate solution of the following millimolar composition (control solution): 118.3 NaCl, 4.7 KCl, 2.5 CaCl$_2$, 1.2 MgSO$_4$, 1.2 KH$_2$PO$_4$, 25 NaHCO$_3$, 0.026 calcium ethylenediamine–tetraacetic acid and 11.1 glucose. Segments, 2–3 mm long, of the left circumflex coronary artery were dissected. When indicated, the endothelium was mechanically removed from the vessels. Each vessel was connected to an isometric force transducer (Grass Instruments, West Warwick, RI, USA) and suspended in an organ chamber filled with 25 ml of control solution (37°C; pH 7.4) and gassed with 94% O$_2$ and 6% CO$_2$. Isometric tension was recorded continuously. The arteries were allowed to stabilize at a resting tension for 1 h. Viability of the vessels was confirmed by a contractile response to 20 mM KCl at baseline, at 2 g, at 4 g and at 6 g, each time after the potassium had been washed out. At 6 g, all vessels were then exposed to 10$^{-6}$ M substance P (Sigma, St. Louis, MO, USA), an endothelium-dependent vasodilator, to verify the functional integrity of the vascular endothelium. All chambers were then washed out using the control solution.

After an equilibration period of 30 min, epicardial arteries were contracted with cumulative concentrations of either 10$^{-10}$–10$^{-6}$ M ET-1 (Phoenix Pharmaceuticals, Mountainview, CA, USA) or 10$^{-11}$–10$^{-7}$ M sarafotoxin 6c (S6c; Phoenix Pharmaceuticals), a selective ETB-receptor agonist [36]. In certain experiments, ET-1 or S6c were added after 20 min of incubation with either 10$^{-8}$ M regular insulin (Eli Lilly, Indianapolis, IN, USA) or IGF-I (Sigma). These concentrations of insulin and IGF-I were derived from preliminary experiments in which epicardial arteries were first contracted with ET-1 (10$^{-7}$ M) and then relaxed with cumulative concentrations of either insulin (n=4 vessels) or IGF-I (n=4 vessels). The EC$_{50}$ for both peptides was found to be approximately 10$^{-8}$ M.

To verify that the effect of insulin and IGF-I on the ET axis is unique, the above experiments were repeated using 10$^{-10}$ to 10$^{-6}$ M U46619 (Cayman Chemicals, Ann Arbor, MI, USA), a thromboxane A$_2$ analog that causes contraction of coronary arteries.

Additional experiments were conducted to examine the vasorelaxing effects of insulin and IGF-I on porcine coronary arteries. Epicardial arteries with intact endothelium were first contracted with 10$^{-7}$ M ET-1 and, after equilibration for 20 min, they were relaxed with cumulative concentrations of either 10$^{-12}$ to 10$^{-7}$ M insulin or 10$^{-12}$ to 10$^{-7}$ M IGF-I. In preliminary experiments, ET-1 at this dose produced sustained contraction of porcine coronary vessels. These experiments were repeated after 20 min of pre-incubation with 10$^{-6}$ M diltiazem (Sigma) prior to contraction with ET-1, in order to examine if the coronary vasorelaxing effects of insulin and IGF-I are through the regulation of calcium influx. In porcine coronary arterial strips, diltiazem at this dose has been shown to inhibit increases in intracellular calcium and tension development induced by cumulative applications of extracellular calcium during potassium-induced contraction [37].

Stock solutions of each agent were prepared every day. Drugs were dissolved in distilled water such that volumes of <0.2 ml were added to the organ chambers. All concentrations are expressed as the concentration within the bath solution. At the end of all experiments, 10$^{-3.5}$ M papaverine (Sigma) was added to verify that the vessels maintained vasodilating capacity.

2.3. Data analysis

Results are presented as mean±SEM. In experiments designed to examine the contractile responses, the contraction attained with 60 mM KCl for each vessel at baseline was considered as 100% contraction. Subsequent measurements of coronary artery contraction are expressed as a percent relative to the contraction attained with KCl. In all experiments, n refers to the number of vessels. In experiments designed to examine the vasorelaxing effects of insulin and IGF-I, the contraction attained with ET-1 for each vessel at baseline was considered as baseline (0% relaxation). Subsequent measurements of coronary artery relaxation are expressed as a percent reduction in contrac-
tion (the maximal relaxation attained with papaverine being 100% relaxation). Experiments were performed in parallel to preclude a situation whereby all vessels in one experiment were harvested from only one animal (on average, each experiment was conducted using vessels from three–four animals). For statistical analysis, ANOVA or repeated measure ANOVA followed by Bonferroni’s t-test were used. A two-tailed p value of ≤0.05 was considered to be significant.

3. Results

3.1. Vessel integrity after endothelium removal

Substance P caused complete vasorelaxation after 20 mM KCl-induced vasoconstriction in endothelium-intact vessels, but did not cause any vasorelaxation in endothelium-removed vessels. The mean response to 60 mM KCl in each protocol is presented in Table 1. Although the maximal response to 60 mM KCl was greater for endothelium-intact than endothelium-removed vessels (6.2±0.3 g vs. 4.4±0.3 g for endothelium-intact and -removed vessels, respectively, p<0.001), the response to KCl was similar in vessels incubated with vehicle, insulin or IGF-I.

3.2. Endothelin-1

The contractile response to ET-1 was greater in vessels without endothelium at 10⁻⁸.⁵–10⁻⁶.⁵ M ET-1: The maximal contraction to ET-1 in vessels with and without endothelium were 158±8 and 200±21%, respectively (p<0.05 at 10⁻⁸.⁵–10⁻⁶.⁵ M). The EC₅₀ values for the contractile response to ET-1 for endothelium-intact and endothelium-removed vessels were 10⁻⁸.⁶ M and 10⁻⁸.⁰ M, respectively (p=0.03). In vessels with intact endothelium, both insulin and IGF-I attenuated the response to ET-1 (Fig. 1), without affecting the EC₅₀. Similarly, in vessels without endothelium, the response to ET-1 was attenuated by insulin and IGF-I (Fig. 2), without affecting the EC₅₀.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Endothelin-1 (g)</th>
<th>Sarafotoxin-6c (g)</th>
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</thead>
<tbody>
<tr>
<td><strong>Intact endothelium</strong></td>
<td></td>
<td></td>
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<tr>
<td>Incubation with vehicle</td>
<td>6.4±0.7</td>
<td>6.4±0.7</td>
</tr>
<tr>
<td>Incubation with insulin</td>
<td>7.8±0.7</td>
<td>4.7±0.9</td>
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<tr>
<td>Incubation with IGF-I</td>
<td>8.3±1.0</td>
<td>5.4±0.5</td>
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<tr>
<td><strong>Without endothelium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubation with vehicle</td>
<td>3.7±0.7</td>
<td>5.4±0.5</td>
</tr>
<tr>
<td>Incubation with insulin</td>
<td>3.8±0.6</td>
<td>3.9±0.5</td>
</tr>
<tr>
<td>Incubation with IGF-I</td>
<td>4.2±0.7</td>
<td>5.2±0.9</td>
</tr>
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Results are presented as grams of tension.

3.3. Sarafotoxin 6

The contractile response to S6c was significantly greater in vessels without the endothelium: The maximal contractions to S6c in vessels with and without endothelium were 54±13 and 84±7%, respectively (p<0.05 at 10⁻⁹, 10⁻⁸.⁵ and 10⁻⁷ M). In a similar manner to that with ET-1, the EC₅₀ values for the contractile response to S6c for endothelium-intact was greater than for endothelium-removed vessels: 10⁻⁸.⁸ and 10⁻⁹.² M, respectively (p=0.04). Insulin and IGF-I did not attenuate the contractile response to S6c in vessels with (Fig. 3) and without endothelium (Fig. 4), nor did they affect the EC₅₀ values in the respective experiments.

3.4. U46619

Porcine epicardial arteries with intact endothelium (n = 6) exposed to the thromboxane A₂ analog U46619 con-
tracted in a concentration-dependent manner (data not shown). The EC$_{50}$ for the contractile response to U46619 was $10^{-7.4}$ M. Insulin ($n=6$) and IGF-I ($n=6$) did not affect the contractile response to U46619 ($p=0.95$ and 0.92 for insulin and IGF-I, respectively; Fig. 5). The EC$_{50}$ values remained similarly unchanged ($p=0.77$ and 0.51, respectively).

3.5. Vasorelaxing effects of insulin and IGF-I

Porcine epicardial arteries with intact endothelium were contracted with ET-1 and then exposed to cumulative concentrations of either insulin ($n=7$) or IGF-I ($n=8$). Both insulin and IGF-I caused a significant decrease in coronary epicardial tension (relaxation of $28.4 \pm 25.3\%$ with insulin and IGF-I, respectively, $p<0.0001$ for each peptide). There was no significant difference in the vasorelaxation response attained with both agents. Pre-incubation of the vessels with diltiazem ($10^{-6}$ M, $n=6$ for each peptide) had no effect on the vasorelaxing effects of either peptide (Fig. 6).

4. Discussion

The principal finding of the current study was that both insulin and IGF-I attenuated ET-1-induced but not S6c-induced coronary epicardial contraction in vitro. Due to the similar effects attained in vessels with and without endothelium, the effects of insulin and IGF-I were endothelium-independent. The interaction between the ET and insulin/IGF pathways has previously focused predominantly on the effect of each pathway on the production, secretion and the effect of their interplay in promoting cell proliferation [30]. Our findings extend the current paradigm regarding this interaction, demonstrating that the vasoactive effects of ET may be regulated by insulin or IGF-I. The lack of inhibition by either peptide of thromboxane-induced porcine epicardial contraction, and more so the selective inhibition of ET-1-induced but not S6c-induced coronary contraction, indicate that this interaction is specific for the ETA-receptor-mediated pathway. Moreover, the endothelium-independence of this interaction points to the coronary vascular smooth muscle cell as the primary locus. Insulin and IGF-I may thus function as endogenous modulators of ET, a potent vasoconstrictor implicated in the pathogenesis of several pathophysiologic conditions [1,2].

4.1. Possible mechanisms

ET-1 increases porcine coronary artery smooth muscle calcium biphasically [34,38]: First, it causes the release of calcium from intracellular stores and then it induces calcium influx via voltage-gated potassium channels. Dick and Sturek [34] have demonstrated that physiological concentrations of insulin blunt the ET-1-induced rise in porcine coronary artery smooth muscle cell calcium, perhaps by modulating transmembrane signal transduction. These authors [34] speculated that insulin’s attenuating
effect is not specific for ET-1, but rather is an effect exerted on all inositol 1,4,5-trisphosphate-mediated vas- 
constrictor agonists.

ETB-receptor stimulation, similarly to ETA-receptor stimulation [34,38,39], can cause an increase in intracellular calcium [40], via stimulation of inositol 1,4,5-trisphosphate [41]. Because our results indicate that the interaction between the ET and insulin/IGF pathways is limited to activation of the ETA receptor, this interaction is probably unique and does not represent an indiscriminate attenuation of vasoconstrictors by insulin and IGF-I, as proposed by Dick and Sturek [34].

ET binding to vascular smooth muscle ETA and ETB receptors mediates vasoconstriction, whereas ETB receptors on the vascular endothelium mediate a vasodilator response, presumably through increased production and release of nitric oxide and/or prostacyclin [42], and activation of potassium channels [2]. Indeed, in our studies, removal of the endothelium resulted in increased contraction to S6c and ET-1. However, in vessels without endothelium, insulin and IGF-I did not attenuate the vascular smooth muscle ETB-receptor-mediated response to S6c, further supporting a unique interaction between the IGF axis and the vascular smooth muscle ETA receptor.

Moreover, Seo et al. [43] previously demonstrated that the contractile response of porcine epicardial arteries to endothelins is biphasic; the first phase attained at low concentrations is primarily mediated by the endothelin ETB receptor, whereas both endothelin receptors mediate contraction in the latter, more pronounced, phase attained at high concentrations. We have confirmed these findings in porcine coronary arterioles [44]. It is thus of interest that, in our current studies, both insulin and IGF-I attenuated the contractile response to high concentrations of ET-1 (i.e., mediated by both ETA and ETB receptors) but not to low concentrations (i.e., mediated primarily by the ETB receptor).

The mechanisms underlying the attenuation of ET-1-induced contraction by insulin and IGF-I may be specu- 
lated. High concentrations of ET-1 (>10 nM) irreversibly inhibit porcine coronary artery smooth muscle cell calcium-activated potassium channels in vitro [45]. Others have reported a similar effect in isolated guinea-pig ileum [46]. By inhibiting these channels, coronary tone is enhanced, resulting in vascular contraction. The in vitro interaction between endothelins and porcine coronary artery smooth muscle cell calcium-activated potassium channels has recently been shown to be mediated solely by the ETA receptor and not by the ETB receptor [47]. Insulin and IGF-I may attenuate the vasoconstrictor effects of ET-1 by activating these channels, in a similar way to their effects on other types of potassium channels [48].

Our studies demonstrating the lack of effect of diltiazem on the vasorelaxing effects of insulin and IGF indicate that these peptides may affect ET-1-induced contraction through the regulation of intracellular calcium stores rather than through the regulation of calcium influx into the vascular smooth muscle cell. As mentioned above, ET-1-induced contraction of porcine coronary arteries initially results in the release of calcium from intracellular pools [34,38]. Insulin and IGF-I may blunt this rise in intracellular calcium. However, because insulin and IGF-I failed to attenuate the contractile response of another potent agent that causes the release of calcium from the intracellular stores, U46619, the interaction between the ET-1 and insulin/IGF axes is probably more complex than just the regulation of intracellular calcium kinetics.

4.2. Concentration of insulin and IGF-I

In our experiments, we used supraphysiological concentrations of insulin and IGF-I to attenuate the vasocon- 
strictor effects of ET-1. Unlike insulin, which is only produced in the liver, IGF-I is produced by various cell types, including endothelial [49,50] and vascular smooth muscle cells [51–53]. IGF-I may therefore serve as an endogenous paracrine/autocrine factor in the regulation of regional vascular tone in physiological and
pathophysiologic states. Because the local concentrations of IGF-I may be higher than the circulating levels, the results of our studies using IGF-I are probably more physiologically pertinent.

5. Conclusion

Insulin and IGF-I attenuated ETA-receptor-mediated coronary contraction through an endothelium-independent mechanism. The IGF axis may serve as an endogenous regulator of endothelium-mediated vasoconstriction.

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