Scientific Contributions

Human Insulin-Mediated Enhancement of Vascular β-Adrenergic Responsiveness

Robert Gros, Kazimierz R. Borkowski, Ross D. Feldman

Abstract  Insulin may play an important role in the physiological and/or pathophysiological regulation of the cardiovascular system. Insulin augments sympathetic activity, increases renal sodium retention, and increases forearm blood flow. Furthermore, hypoglycemia and insulin resistance have been associated with elevated blood pressure. However, a causal relation has yet to be established.

Recent studies have focused on the role of insulin as an endogenous vasodilator. In humans, insulin increases blood flow and reduces vascular resistance despite augmentation of sympathetic activity. Further insulin-mediated increases in blood flow were suggested to occur via a β-adrenergic mechanism (ie, were blocked by β-adrenergic receptor antagonists) in both canine and human studies. However, the mechanism for this effect remains unexplained. Therefore, to determine whether insulin might have a role in regulating vascular responsiveness through alterations in adrenergic responsiveness, we examined the effect of insulin on tension development in isolated rat thoracic aortic ring segments.

Methods

Drugs

Phenylephrine hydrochloride, (+)-isoproterenol hydrochloride, sodium nitroprusside, and acetylcholine chloride were obtained from Sigma Chemical Co. Human insulin was obtained from Eli Lilly & Co. All standard laboratory chemicals were obtained from BDH.

Animals

Male SHR and normotensive Wistar rats (200 to 300 g, Charles River) were used throughout. The rats were cared for in accordance with Canadian Council on Animal Care guidelines and housed under a 12-hour light/dark cycle, with free access to standard laboratory rat chow and drinking water.

Assessment of Tension in Aortic Ring Segments

Assessment of tension in aortic ring segments was performed according to our previously published methods. Briefly, rats were anesthetized with 5% halothane in a mixture of 50% nitrous oxide and 50% oxygen using a Harvard rat tail blood pressure monitor (Ealing).

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Contributions

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Results

The addition of phenylephrine to isolated ring segments from Wistar rats resulted in an initial rapid development of tension and sustained steady plateau (Fig 1). The addition of isoproterenol to maximally phenylephrine-preconstricted rings resulted in a rapid relaxation, reaching a nadir by 4 minutes followed by a gradual, partial recovery of tension that remained stable for more than 30 minutes (Fig 1).

We considered whether the partial recovery of tension after isoproterenol addition might be due to degradation of the drug. However, a second addition of isoproterenol 20 minutes after the initial addition did not significantly augment the relaxation response (42.7±3.6% of maximal tension before second addition of isoproterenol to 36.1±2.9% of maximal tension after second addition, n=4; P=.15 for paired observations). Additionally, for direct assessment of the stability of isoproterenol degradation, samples were taken after drug addition, and bath concentrations were assessed by high-performance liquid chromatography. No significant reductions in organ bath isoproterenol concentration were evident throughout the duration of the experiment (Fig 2).

To determine whether insulin altered α-adrenergic vasoconstriction in isolated ring segments, phenylephrine-mediated constriction was examined. Insulin (0.01 to 10.0 nmol/L) exposure did not affect phenylephrine-mediated constriction (Fig 3).

To assess whether insulin altered β-adrenergic-mediated vasorelaxation, we examined the effects of isopro-

![Fig 1. Transcription of a representative tracing performed under baseline conditions shows effect of phenylephrine (3 μmol/L) and isoproterenol (10 μmol/L) on tension in thoracic ring segments. Shaded area represents area below the phenylephrine constriction curve; hatched area represents area above the isoproterenol relaxation curve.](image1)

![Fig 2. Line graph shows stability of isoproterenol after addition to organ bath determined in samples taken at 1, 2, 4, 6, 8, 10, and 20 minutes after addition of isoproterenol (10 μmol/L). Values represent mean±SEM of four replicate samples per time point each from three separate experiments.](image2)

![Fig 3. Bar graph shows effect of insulin on phenylephrine-mediated constriction in isolated ring segments. Values are expressed as percentage change from baseline phenylephrine-mediated constriction and represent mean±SEM; n=5 to 15 at each insulin concentration.](image3)
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Insulin (0.01 to 10.0 nmol/L) was associated with a concentration-dependent increase in the extent of isoproterenol-mediated vasorelaxation in phenylephrine-precontracted rings (Figs 4 and 5). The EC₅₀ for the insulin effect was 32 pmol/L, with a maximum of 20±4% above baseline isoproterenol-mediated relaxation (n=12, P<.001). As noted above, isoproterenol-mediated relaxation followed a biphasic response, with an initial maximal phase of relaxation (up to 4 minutes after addition) followed by a partial recovery of tension that then remained stable throughout the test period. To further assess the role of insulin on β-adrenergic responses, we considered isoproterenol-mediated relaxation during the “onset” phase (0 to 4 minutes) and “recovery” phase (4 to 20 minutes). As shown in Table 1, insulin-mediated augmentation of isoproterenol-mediated response was accounted for (primarily) by enhancement of relaxation during the recovery phase of the response (to a maximum of 29±7%, with an EC₅₀ of 25 pmol/L).

We next examined the role of the endothelium in both isoproterenol-mediated relaxation and its enhancement by insulin. After endothelium removal, maximal isoproterenol-mediated relaxation (in the absence of insulin) was unaltered (nadir effect, endothelium-denuded rings: 30.7±7.7% of maximal tension, n=6; endothelium-intact rings: 28.2±4.1% of maximal tension, n=17; P=.77). However, the extent of the “rebound” recovery in tension was exaggerated in endothelium-denuded rings (effect at 20 minutes, endothelium-denuded rings: 77.8±4.8% of maximal tension; endothelium-intact rings: 60.5±4.5% of maximal tension; P=.04). In addition, overall isoproterenol-mediated relaxation during the recovery phase, as derived from area above the curve calculations from 4 to 20 minutes, was decreased in endothelium-denuded ring segments (Table 2). Furthermore, in endothelium-de-nuded rings, human insulin (0.1 to 10.0 nmol/L) did not significantly enhance isoproterenol-mediated relaxation (Fig 6).

To assess whether insulin-mediated enhancement of β-adrenergic-mediated relaxation reflected a generalized enhancement in vasorelaxation, we also examined sodium nitroprusside-mediated relaxation. The addition of sodium nitroprusside to maximally phenylephrine-precontracted ring segments (in the absence of insulin) resulted in a rapid relaxation, reaching a nadir by 4 minutes (to 15.0±4.2% of maximal tension, n=6) followed by a gradual, partial recovery of tension (effect at 20 minutes after addition, 27.6±10.7% of maximal tension). Insulin (0.01 to 10.0 nmol/L) did not significantly affect sodium nitroprusside-mediated relaxation in phenylephrine-precontracted rings (Fig 7).

The extent of human insulin on isoproterenol-mediated relaxation was examined in rings obtained from age-matched SHR maintained under the same conditions as the Wistar rats. At the time of study, systolic blood pressures (determined with tail-cuff techniques) were significantly higher in SHR compared with those measured in concurrently studied Wistar rats (142±3 versus 90±2 mm Hg, SHR [n=10] versus Wistar [n=5]; P<.001). The extent of phenylephrine-mediated tension developed was marginally increased in Wistar rats compared with those obtained in SHR (550±35 versus 469±29 mg, Wistar [n=30 rings] versus SHR [n=20 rings]; P=.08 by Welch’s t test for two populations with unequal variances).

In phenylephrine-precontracted SHR aortic ring segments, addition of isoproterenol resulted in an initial relaxation (reaching a nadir by 4 minutes of 48.8±4.1% of maximal tension, n=10) followed by a partial recovery of tension (effect at 20 minutes after addition, 73.9±1.9% of maximal tension). Although the pattern of relaxation and rebound in SHR aortic ring segments was qualitatively similar to that observed in Wistar rat segments, the extent of both nadir relaxation and relaxation after partial recovery was significantly attenuated in SHR compared with Wistar rats (nadir, P=.0001, and recovery, P=.009 by Welch’s t test versus the effects of isoproterenol on Wistar rings). As in ring segments from Wistar rats, insulin did not affect the extent of phenylephrine-mediated relaxation.

TABLE 1. Effect of Insulin on Onset and Recovery Phases of Isoproterenol in Wistar Thoracic Aortic Ring Segments

<table>
<thead>
<tr>
<th>Insulin, nmol/L</th>
<th>Onset, %</th>
<th>Recovery, %</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>102±2</td>
<td>107±7</td>
<td>5</td>
</tr>
<tr>
<td>0.1</td>
<td>108±2</td>
<td>122±6*</td>
<td>15</td>
</tr>
<tr>
<td>1.0</td>
<td>104±3</td>
<td>122±5*</td>
<td>14</td>
</tr>
<tr>
<td>10.0</td>
<td>109±3*</td>
<td>129±7*</td>
<td>12</td>
</tr>
</tbody>
</table>

Data are expressed as percentage of baseline isoproterenol-mediated relaxation and represent mean±SEM. n indicates number of experiments. *P<.05 vs baseline isoproterenol-mediated relaxation by one-way ANOVA.
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mean±SEM; n=6 at each insulin concentration. ND indicates not determined.

Table 2. Comparison of Area Above the Curve During Overall, Onset, and Recovery in Endothelium-Intact and Endothelium-Denuded Wistar Thoracic Aortic Ring Segments

<table>
<thead>
<tr>
<th>Condition</th>
<th>Overall</th>
<th>Onset</th>
<th>Recovery</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelium-intact</td>
<td>1012±78</td>
<td>243±12</td>
<td>799±67</td>
<td>17</td>
</tr>
<tr>
<td>Endothelium-denuded</td>
<td>785±109</td>
<td>238±24</td>
<td>547±89</td>
<td>6</td>
</tr>
</tbody>
</table>

P<.076 P=.8 P=.018

Area above the curve is expressed as percent tension x minute and represents mean±SEM. n indicates number of experiments. Statistical analysis was by one-way ANOVA. P value is for comparison of responses in endothelium-intact vs endothelium-denuded groups.

Discussion

In the present study we demonstrated that acute exposure to insulin selectively enhances β-adrenergic responsiveness in aortic ring segments from Wistar rats but not in aortas obtained from SHR. Furthermore, the observed response to insulin was dependent on the presence of a functionally intact endothelium.

Isoproterenol-mediated relaxation in phenylephrine-constricted rings followed two distinct phases. A peak relaxation phase was followed by a phase characterized by partial recovery of tension that then remained stable throughout the observation period. A biphasic response to isoproterenol, similar to that observed in our studies, has been previously reported in ring segments from rabbit aortas.10 The authors of that study concluded that the recovery of tension was due to desensitization of the β-adrenergic receptor and not to the degradation of isoproterenol. Consistent with this conclusion, our results indicate that the loss of relaxant response was not due to isoproterenol degradation. Furthermore, the lack of any augmentation of the relaxant response after a second addition of isoproterenol is consistent with the hypothesis that the recovery in tension is due to desensitization. Additionally, the extent of attenuation and the time course for this process parallel the time course for in vitro desensitization of β-adrenergic-mediated adenylcyclase activity observed in cell model systems.11-13 Notably, a rebound in tension was also noted after nitroprusside-mediated relaxation (although to a lesser extent than that observed after isoproterenol administration). Previous investigators have ascribed this effect to desensitization of guanylyl cyclase activation.14,15

Insulin exposure selectively enhances isoproterenol-mediated relaxation without altering phenylephrine-mediated constriction or nitroprusside-mediated relaxation. Previous studies have reported variable effects of insulin on α-adrenergic-mediated vasoconstriction. High insulin levels have been associated with increased responsiveness in rat mesenteric vasculature16 and decreased responsiveness in rat mesenteric arterioles17 and rabbit femoral arteries and veins.18 The explanation for these differences is unclear but could reflect differences in species, vascular beds, type of insulin, or duration of insulin exposure. It is notable that in our study the effect of insulin was assessed without a period of pretreatment, whereas previous studies16-18 used longer durations of preexposure (30 to 90 minutes).

The effect of insulin was most pronounced in the later phase of isoproterenol-mediated relaxation, the stage characterized by a partial recovery of tension. As discussed above, previous authors, observing a similar phenomenon in rabbit aortic ring segments, ascribed it to β-adrenergic desensitization. Our findings would support this hypothesis. If the partial recovery of tension with prolonged isoproterenol exposure does represent β-adrenergic desensitization, the effect of insulin in enhancing β-adrenergic response could be via attenua-

![Fig 6](image6.png)  
Fig 6. Bar graph shows effect of varying insulin concentrations on isoproterenol-mediated relaxation in endothelium-denuded ring segments. Values are expressed as percentage change from baseline isoproterenol-mediated relaxation and represent mean±SEM; n=6 at each insulin concentration. ND indicates not determined.

![Fig 7](image7.png)  
Fig 7. Bar graph shows effect of varying insulin concentrations on sodium nitroprusside-mediated relaxation and represent mean±SEM; n=6 at each insulin concentration.

![Fig 8](image8.png)  
Fig 8. Bar graph shows effect of 10 nmol/L insulin on isoproterenol-mediated relaxation in ring segment from spontaneously hypertensive rats (SHR) compared with effect of insulin on ring segments from Wistar rats. Values are expressed as percentage change from baseline isoproterenol-mediated relaxation and represent mean±SEM; n=10 for SHR and n=12 for Wistar rats. *P<.05 comparing change in isoproterenol-mediated relaxation in SHR vs Wistar rats.
Enhancement of β-adrenergic responsiveness after insulin exposure was not observed in tissues obtained from SHR. Vascular β-adrenergic responsiveness is impaired in hypertensive animals (reviewed in Reference 23). Additionally, impaired endothelium-mediated relaxation has been reported in SHR. Furthermore, SHR have been reported to be insulin resistant.26 Therefore, whether the lack of any detectable enhancement of β-adrenergic responsiveness by insulin in SHR is due to impaired β-adrenergic-mediated effects, impaired endothelium-mediated relaxation, impaired insulin-mediated responses, or a combination of these remain to be determined. However, regardless of the mechanism, if insulin-mediated sensitization of β-adrenergic-mediated vasodilation occurs in vivo and is important in the maintenance of normal peripheral resistance, then an impairment in this mechanism could contribute to the defect in β-adrenergic-mediated vasodilation that occurs in both human and animal models of hypertension.

Acknowledgments

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References


### Table 3. Effect of Insulin on Overall, Onset, and Recovery Phases of Isoproterenol in Thoracic Aortic Ring Segments From Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Insulin, nmol/L</th>
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<th>Onset, %</th>
<th>Recovery, %</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>0.01</td>
<td>93±10</td>
<td>101±11</td>
<td>92±10</td>
<td>6</td>
</tr>
<tr>
<td>0.1</td>
<td>110±11</td>
<td>106±9</td>
<td>111±12</td>
<td>10</td>
</tr>
<tr>
<td>1.0</td>
<td>101±12</td>
<td>94±9</td>
<td>105±14</td>
<td>10</td>
</tr>
<tr>
<td>10.0</td>
<td>99±8</td>
<td>92±6</td>
<td>91±9</td>
<td>10</td>
</tr>
</tbody>
</table>

Data are expressed as percentage of baseline isoproterenol-mediated relaxation and represent mean±SEM. n indicates number of experiments.
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