Insulin Enhances Pressor Responses to Norepinephrine in Rat Mesenteric Vasculature


Hyperinsulinemia and insulin resistance have been proposed to play a role in human and experimental hypertension. To characterize this relation further, we examined the pressor responses to periarterial nerve stimulation (PNS) and norepinephrine infusion in the isolated mesenteric vasculature of the normal rat before and after insulin (10, 100, and 1,000 microunits/ml) infusion. The pressor responses to PNS were similar before and after insulin, except at the highest dose of insulin (1,000 microunits/ml) and the highest frequency of PNS (10 Hz). However, insulin significantly increased the pressor responses to norepinephrine. This increase in responsiveness was evident at all doses of insulin studied. In contrast, insulin did not affect the pressor responses to either angiotensin II or serotonin administration. The mechanism or mechanisms for the augmented pressor response to norepinephrine after insulin infusion remain to be determined. However, the selectivity of the response for norepinephrine and the absence of a marked pressor increase after PNS indicate that the mechanism probably involves either the α-receptor itself or an amplification of the postreceptor signal transduction. The role of chronic hyperinsulinemia and insulin resistance in the pathogenesis of essential hypertension requires further study. (Hypertension 1992;19[suppl II]:II-105–II-110)
Insulin Administration

The time required for PNS and norepinephrine infusion was 40 minutes. After this, insulin was added to the perfusion buffer to achieve final concentrations of 10 (n=6), 100 (n=6), and 1,000 (n=6) microunits/ml. No albumin was added, because aeration of perfusion buffer causes frothing, impeding buffer flow through the vasculature. After 90 minutes of insulin perfusion, PNS and norepinephrine responses were determined again, while the insulin perfusion continued.

Norepinephrine, Angiotensin II, and Serotonin Responses

In a final set of experiments, the effects of the highest concentration of insulin (1,000 microunits/ml) on vasoconstriction mediated by norepinephrine (n=12), angiotensin II (n=10), and serotonin (n=10) were determined. In all mesenteric vascular preparations, initial pressor responses to PNS (4, 6, 8, and 10 Hz) and to a 10-second perfusion (10 µl) of 4 nmol norepinephrine, 800 pmol angiotensin II, or 8 nmol serotonin were determined. To ensure that changes in perfusion pressure were not due to instability of the mesenteric preparation over time, each series of vasoconstrictor administration had two groups. A control group received a buffer infusion without any insulin during the period of time between the baseline responses and the determination of pressor responses to a second series of PNS and vasoconstrictor administration. A second group, the experimental group, received an infusion of buffer with insulin (1,000 microunits/ml) between the baseline pressor responses and the second set of pressor determinations. As before, perfusion pressure changes were recorded on a strip chart. After the baseline PNS and vasoconstrictor responses, the buffer (with or without insulin added) infusion continued for an additional 90 minutes. At the end of 90 minutes, PNS responses were again determined, followed by an identical bolus of vasoconstrictor. The duration of each vasoconstrictor administration was 10 seconds in each study.

Norepinephrine Overflow

An internal standard of 3,4 dihydroxybenzylamine hydrobromide (DHBA) (40 ng) was added to each collection tube during PNS stimulation. Perfusate samples were collected for a total of 2 minutes each (during the 30 seconds of PNS and for the next 1.5 minutes) and were deproteinized with 4 ml of 1N perchloric acid; the catecholamines were absorbed on 200 µg acid-washed alumina in a 1 M ammonium acetate buffer at pH 8.5 for 10 minutes with constant stirring. The alumina was washed with 10 ml distilled water and aspirated. After spinning to dryness, the catecholamines were eluted from the alumina with 400 µl of 0.1N perchloric acid. The amount of norepinephrine in each sample was determined by high-performance liquid chromatography using previous methods.8

Pharmacological Agents

The following agents were used: regular insulin (Humulin, Eli Lilly Co., Indianapolis, Ind.), l-norepinephrine bitartrate (Sigma Chemical Co., St. Louis,
Mo.), angiotensin II (human, octapeptide, Bachem Inc., Torrance, Calif.), and serotonin (Sigma). Drugs were first dissolved in distilled water and then diluted in the aerated buffer solution.

**Data Analysis**

Values are expressed as mean±SEM. Data were analyzed for statistical significance using two-way and three-way analysis of variance with post hoc mean comparison using the Newman-Keuls multiple-range test. Student's t test for group mean comparisons was used when only two means were compared.

**Results**

**Responses to Periarterial Nerve Stimulation and Norepinephrine Before and After Insulin Infusion**

The mean perfusion pressure for this entire group (n=18) was 21.3±0.4 mm Hg. Pressor responses to PNS and norepinephrine were determined before and after 10, 100, and 1,000 microunits/ml insulin infusion. These responses are shown in Figures 1 and 2 (n=6 for each of the three doses of insulin), and the data are expressed as the change in perfusion pressure (above baseline) for each intervention (PNS and norepinephrine infusion). There was no significant change in basal perfusion pressure after insulin infusion. There was no significant difference in the PNS response after insulin infusion at any dose, with the exception of the highest dosage of insulin (1,000 microunits/ml) at the highest frequency (10 Hz) of stimulation (p<0.01) as shown in Figure 1 (bottom panel). Insulin administration significantly increased the pressor response to norepinephrine. The 10-microunits/ml dosage of insulin significantly increased the pressor response to 8 nmol norepinephrine. Both the 100- and 1,000-microunits/ml dosages of insulin significantly increased the pressor responses to both 4 and 8 nmol norepinephrine administration. The highest dose of insulin tended also to increase the pressor response to the lowest (2 nmol) dose of norepinephrine (Figure 2).

**Norepinephrine Overflow**

Only results from perfusion with insulin (1,000 microunits/ml) and the highest frequency of PNS (10 Hz) are reported here. Preinsulin release of norepinephrine during PNS at 10 Hz was 32.6±0.6 ng, and the amount released after insulin administration was 32.1±1.3 ng (n=4; p=NS).

**Norepinephrine, Angiotensin II, and Serotonin Responses Before and After Insulin**

The specificity of the increased norepinephrine pressor response after insulin administration was subsequently evaluated. This was accomplished by determining pressor responses to norepinephrine, angiotensin II, and serotonin before and after exposure to the highest insulin dosage (1,000 microunits/ml). These results are detailed in Figure 3. Data in Figure 3 are expressed as the percentage change in perfusion pressure, with 100% equal to identical vasoconstrictor pressor response before and after administration of either insulin (experimental group) or buffer alone (control group). The pressor response to 4 nmol norepinephrine was significantly higher after insulin administration (n=6, control group [no insulin between responses]; n=6, insulin group). These findings are similar to those seen in the first set of experiments (Figure 2, bottom panel), thus confirming these observations. In contrast to the enhanced pressor responses to norepinephrine, neither angiotensin II (n=4, control; n=6, insulin) nor serotonin (n=5, control; n=5, insulin) demonstrated any augmentation in pressor responses after insulin administration.

**Discussion**

The role of insulin resistance and hyperinsulinemia in blood pressure and regional blood flow...
regulation is an area of recent intense interest. The results of our study demonstrate that insulin administration at and above physiological concentrations enhances the pressor responsiveness of norepinephrine in the isolated intact mesentery of the normal rat. Interestingly, the enhanced pressor responsiveness was more pronounced for norepinephrine than PNS and was more evident at higher (100 microunits/ml) physiological insulin levels, while showing no further augmentation in the pressor response with pharmacological (1,000 microunits/ml) insulin levels. The enhanced pressor responsiveness appears to be selective for norepinephrine, because the pressor responsiveness to exogenously administered angiotensin II or serotonin under the same conditions was not affected by insulin.

PNS produces a frequency-dependent increase in perfusion pressure as demonstrated in this study and in previous work. Collection of the perfusate during PNS responses reveals that the amount of norepinephrine released increases as the frequency increases. However, the amount of norepinephrine released per stimulus is constant in the frequency range (4–10 Hz) used in this study. Thus, enhancement in the pressor response with increasing frequency of PNS is due to the greater total amount of norepinephrine released within the synapse. Because insulin administration did not greatly augment pressor responses to PNS, it is unlikely that insulin causes a significant increase in the amount of norepinephrine released per neural stimulus, as we have shown herein during PNS at 10 Hz at the highest insulin dose.

The pressor response to exogenous norepinephrine infusion is due to the stimulation of intrasynaptic and extrasynaptic a-receptors. Norepinephrine infusion produced a dose-dependent increase in perfusion pressure as the amount of norepinephrine given increased from 2 to 8 nmol. Infusion of insulin significantly enhanced the pressor responsiveness to norepinephrine. This enhancement was seen even at low physiological insulin concentrations (100 microunits/ml) as evidenced by the pressor response to 8 nmol norepinephrine. Much more striking was the pressor response at the 100- and 1,000-microunits/ml infusions of insulin. The enhanced vasoconstriction was specific for norepinephrine, because insulin did not alter the pressor responsiveness to either angiotensin II or serotonin. This implies that insulin, in this model, selectively augments the a-receptor response, either at the level of the a-receptor itself or through changes that amplify signal transduction at the postreceptor level. Although ATP may be coreleased with norepinephrine and stimulate a pressor response through a purinergic receptor in some vasculatures, prior work in this model indicates that the a-receptor is the major component of the pressor response to both PNS and norepinephrine infu-
The failure to significantly augment the perfusion pressure response to PNS poses the question of why the norepinephrine released during PNS was not associated with potentiation of the vasoconstrictor effect. Although insulin did not largely affect the pressor response to PNS, increases in perfusion pressure response at the higher PNS frequencies (8 and 10 Hz) were observed reaching significance at 10 Hz after the 1,000-microunits/ml dosage of insulin infusion. Because PNS primarily affects intrasynaptic norepinephrine receptors and exogenous norepinephrine affects both intrasynaptic and extrasynaptic norepinephrine receptors, the differences between the two may imply a more important pressor role for the extrasynaptic norepinephrine receptors in the insulin effect in our study. Finally, other studies have shown modulation of the amount of norepinephrine released during PNS in hypertension. One study demonstrated that despite identical PNS pressor responses before and after intervention, the amount of norepinephrine released during PNS declined after intervention. The authors concluded that the smaller amount of norepinephrine released produced identical pressor responses due to enhanced receptor vasoconstrictor effects.

Our results in this in vitro preparation stand in contrast to recent in vitro and in vivo studies demonstrating a vasodilator effect of insulin infusion. In vitro studies have demonstrated that insulin administration vasodilates isolated vascular beds, such as rat tail artery. Different vascular beds may respond differently to pressor and dilator neurohumoral stimuli. Thus, the differences between our study and other reports may be due to the different methods used or vascular regions studied.

Studies conducted with the entire circulatory system intact introduce variables such as flow (cardiac output), baroreceptor input, and changes in vasoactive substances. In a recent study, insulin was shown to blunt the forearm vasoconstriction induced by exogenous angiotensin II administration in normotensive subjects. Another study examined forearm hemodynamic responses to phenylephrine before and after insulin administration in human diabetics. There was less decrease in vascular flow in the forearm following phenylephrine infusion after insulin administration, indicating there was less increase in vascular resistance. Thus, insulin appeared to blunt the vasoconstriction of phenylephrine, thereby acting as a vasodilator. It is difficult to compare these studies with ours because they are in vivo studies, whereas ours is in vitro. Other studies indicate that acute insulin infusion is associated with an increased blood pressure and heart rate. However, the changes in systolic blood pressure were more prominent than for diastolic blood pressure, indicating a greater contribution of an increased cardiac output. It is possible that changes in cardiac output that occur in vivo can produce local alterations in vascular resistance. These changes would not occur in our study because constant flow was maintained. In addition, the acute response of a vasoactive agent may differ from the chronic response of that same agent. Such a difference has been proposed for parathyroid hormone, which when administered acutely causes vasodilation but when chronically elevated may cause vasoconstriction.

The effects of chronic hyperinsulinemia on blood pressure have been examined in normal dogs infused with enough insulin to increase fasting levels fourfold to sixfold without hypoglycemia, with no blood pressure rise noted. In a separate study, insulin did not potentiate the vasoconstrictive effect of infused angiotensin II in dogs. However, it is difficult to produce sustained elevations in blood pressure by measures such as vasoactive pressor infusions in the normal state when normal circulatory counterregulatory responses may offset the changes induced by the infusions. In dogs, acute high-dose insulin infusion produces a significant positive inotropic effect. Hypertensive human diabetics receiving insulin showed a reduction in blood pressure levels when insulin dose was lowered without any concomitant change in weight or antihypertensive therapy. Thus, clinical studies indicate that there is evidence for a role of insulin in blood pressure regulation, but less is known about its direct effects on vascular resistance in the absence of changes in blood flow.

The mesenteric loop preparation used in this study allows for examination of hemodynamic effects in a controlled environment. In our study, insulin clearly augments the pressor effect of norepinephrine, and this appears to be specific for norepinephrine. Moreover, the effect of insulin on the norepinephrine pressor response is evident at physiological levels (10–100 microunits/ml) of insulin. Because this preparation is without humoral counterregulatory responses, it is difficult to compare our results with animal and clinical studies in which such responses are intact. In addition, some authors have shown that the route of norepinephrine administration may have hemodynamic importance. Significant differences in the magnitude of mean arterial pressure change, duration of effect, red blood cell velocity, and caliber of arteriole were found when intravenous administration of norepinephrine was compared with direct intra-arterial injection, with pressor effects of an intra-arterial injection of norepinephrine demonstrated in the absence of systemic pressor effects.

In summary, the results of our study demonstrate that insulin specifically increases the pressor responsiveness to norepinephrine in mesenteric circulations. Insulin administration, by itself, did not alter basal perfusion pressure. Although the mechanism is not clear, this increase does not appear to be mediated by increased presynaptic release of norepinephrine, because PNS responsiveness was largely unaffected. The mechanism or mechanisms by which insulin, insulin resistance, or both influence blood pressure remain to be determined.
References


Insulin enhances pressor responses to norepinephrine in rat mesenteric vasculature.
R R Townsend, R Yamamoto, M Nickols, D J DiPette and G A Nickols

Hypertension. 1992;19:II105
doi: 10.1161/01.HYP.19.2_Suppl.II105

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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