Tissue-Specific Response to Interstitial Angiotensin II in Humans

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Abstract—Angiotensin II is synthesized locally in various tissues; however, the role of interstitial angiotensin II in the regulation of regional metabolism and tissue perfusion is not clear. We characterized the effect of interstitially applied angiotensin II in skeletal muscle and subcutaneous adipose tissue of young, normal-weight, healthy subjects by using the microdialysis technique. Furthermore, we tested the hypothesis that the effect of interstitial angiotensin II is modulated by nitric oxide. Intravascular and interstitial angiotensin II concentrations may contribute to essential hypertension. Moreover, Ang II stimulates cell growth and differentiation and exerts metabolic effects that may contribute to cardiovascular disease. Ang II is generated in both the intravascular and interstitial space. The systemic and local constituents of the renin-angiotensin system are more highly than plasma concentrations. In this model, acute ACE inhibition markedly reduced plasma Ang II. In contrast, interstitial Ang II did not change. Similarly, acute volume expansion elicited a differential effect on intravascular and on interstitial Ang II. The fact that Ang II concentrations in the intravascular and in the interstitial space are independently regulated may suggest that Ang II has different physiological effects in both compartments. Intravascular Ang II, for example, elicits a profound increase in vascular tone through stimulation of Ang II type 1 (AT1) receptors. Interstitial Ang II, however, causes a moderate decrease in blood flow in adipose tissue, and this effect is clearly dependent on tissue location. Nevertheless, the physiology of interstitial Ang II in humans is still poorly understood. We sought to elucidate the effect of interstitial Ang II on tissue perfusion, metabolism, and interstitial norepinephrine in skeletal muscle (primarily energy consuming) and in adipose tissue (primarily energy storing). We used the microdialysis technique to apply Ang II locally to the interstitial space. Furthermore, we determined whether or not the effect of interstitial Ang II is modulated by nitric oxide (NO).

Key Words: glucose ▪ lactates ▪ angiotensin II ▪ microcirculation

A ngiotensin (Ang) II has a pivotal role in the regulation of vascular tone and of electrolyte and water homeostasis and may contribute to essential hypertension. Moreover, Ang II stimulates cell growth and differentiation and exerts metabolic effects that may contribute to cardiovascular disease. Ang II is generated in both the intravascular and interstitial space. The systemic and local constituents of the renin-angiotensin system are more highly than plasma concentrations. In this model, acute ACE inhibition markedly reduced plasma Ang II. In contrast, interstitial Ang II did not change. Similarly, acute volume expansion elicited a differential effect on intravascular and on interstitial Ang II. The fact that Ang II concentrations in the intravascular and in the interstitial space are independently regulated may suggest that Ang II has different physiological effects in both compartments. Intravascular Ang II, for example, elicits a profound increase in vascular tone through stimulation of Ang II type 1 (AT1) receptors. Interstitial Ang II, however, causes a moderate decrease in blood flow in adipose tissue, and this effect is clearly dependent on tissue location. Nevertheless, the physiology of interstitial Ang II in humans is still poorly understood. We sought to elucidate the effect of interstitial Ang II on tissue perfusion, metabolism, and interstitial norepinephrine in skeletal muscle (primarily energy consuming) and in adipose tissue (primarily energy storing). We used the microdialysis technique to apply Ang II locally to the interstitial space. Furthermore, we determined whether or not the effect of interstitial Ang II is modulated by nitric oxide (NO).

Methods

Subjects

The study population included 8 drug-free, nonsmoking male volunteers, 29±4 years of age, height of 1.82±0.02 m, and body mass index of 23.62±1.81 kg/m². All were healthy as determined by medical history,
physical examination, and routine blood and urine tests. The study was approved by the Institutional Review Board of Humboldt University, Berlin, and the subjects gave written informed consent.

Microdialysis

After an overnight fast, two microdialysis catheters each (distance, 2.5 cm) were inserted into abdominal subcutaneous adipose tissue (adipose) at the level of the umbilicus and into skeletal muscle (quadriceps femoris, vastus lateralis). Details of that technique are described elsewhere. Before insertion of the catheters, a local anesthetic (lidocaine) was given, either as a cream for adipose tissue, and frozen at -80°C until analysis.

Ang II was added successively to the perfusion medium for testing the dose-response relation of Ang II on baseline blood flow and dialysate concentrations. Ang II and L- and D-NAME were purchased from CMA/600 analyzer (CMA Microdialysis AB). Samples for norepinephrine were processed and analyzed as previously described.14

Calculations and Statistics

Changes in blood flow were determined by using the ethanol dilution technique, which is based on the Fick principle. Accordingly, a decrease in the outflow/inflow ratio is equivalent to an increase in blood flow and vice versa. For simplicity, the term ethanol ratio is substituted for the term ethanol outflow/inflow ratio. Changes in glycerol were used to assess changes in lipolysis and/or lipid mobilization; changes in glucose, lactate, and pyruvate were used to assess changes in carbohydrate metabolism. In situ recovery for ethanol, glucose, lactate, and pyruvate were measured on a CMA/600 analyzer (CMA Microdialysis AB). Samples for norepinephrine were processed and analyzed as previously described.14

Results

Resting heart rate was 56 ± 6 bpm. Resting systolic and diastolic blood pressures were 118 ± 7 and 63 ± 10 mm Hg, respectively. These values did not change significantly during local perfusion of adipose tissue and muscle with Ang II by microdialysis (data not shown).

In a subset of experiments it was tested whether D-NAME, the isoform ineffective for blocking NOS, could elicit any unspecific effects on tissue blood flow and metabolism. Compared with baseline values in the absence of D-NAME, the presence of D-NAME in the perfusate did not influence ethanol ratio and dialysate concentrations of glucose, lactate, pyruvate, and glycerol (data not shown). Therefore, in the following, all baseline values in the presence of D-NAME can be considered as control values.

Blood Flow

Baseline ethanol ratio was ~0.30 and 0.08 in adipose tissue and muscle, respectively, without any significant differences between L- and D-NAME (Figure 1). Ang II did not result in any

Figure 1. Changes in ethanol ratio and dialysate glucose, lactate, and glycerol concentrations. Abdominal subcutaneous adipose tissue and skeletal muscle were perfused with Ringer's solution + EtOH and increasing doses of Ang II supplemented in the final perfusion step with NP (4 mmol/L) in the presence of either L- or D-NAME (100 μmol/L), using the microdialysis technique. Data are given as mean ± SEM, n = 8; Ang II did not affect ethanol ratio and dialysate glucose and lactate in adipose tissue and muscle. Ang II induced a dose-dependent increase in dialysate glycerol in adipose tissue but a decrease in muscle. *P < 0.05 when compared with baseline for D- and L-NAME, respectively.
significant change in ethanol ratio in both tissues, neither with L-nor with D-NAMe. Final addition of NP to the medium induced a significant decrease in ethanol ratio down to ~0.15 and 0.04 in adipose tissue and muscle, respectively (Figure 1).

**Metabolites**

Baseline dialysate concentration of glucose was ~0.96 and 1.90 mmol/L, whereas that of lactate was ~0.55 and 0.95 mmol/L in adipose tissue and muscle, respectively (L-NAMe=D-NAMe). There were no significant changes in the concentrations of glucose and lactate during Ang II perfusion, either in adipose tissue or in muscle (Figure 1). However, dialysate glucose concentration increased significantly to ~1.7 mmol/L in adipose tissue and 2.6 mmol/L in muscle after NP administration (Figure 1). In both tissues, dialysate lactate concentration was not affected by NP (Figure 1). The response of dialysate glycerol to Ang II showed tissue-specific differences. In adipose tissue, dialysate glycerol increased dose-dependently from 115±9 and 100±12 μmol/L to 149±12 and 131±13 μmol/L in the presence of L- and D-NAMe, respectively (P=0.0438, Figure 1). At all Ang II concentrations used, dialysate glycerol concentrations were higher in the presence of L-NAMe compared with D-NAMe; however, the difference did not reach a significant value (P=0.0531). The relative changes in dialysate glycerol did not differ between L- and D-NAMe. Addition of NP was followed by a decrease in dialysate glycerol down to ~50 μmol/L (L-NAMe=D-NAMe). In muscle, Ang II induced a dose-dependent decrease in dialysate glycerol from ~34 to 22 μmol/L (P<0.007), with no differences between L-NAMe and D-NAMe (Figure 1). After addition of NP, dialysate glycerol returned back to baseline level. Dialysate pyruvate increased dose-dependently during Ang II perfusion in muscle from ~0.20 to 0.045 mmol/L (P=0.0002, L-NAMe=D-NAMe) (Figure 2). Correspondingly, the lactate/pyruvate ratio decreased dose-dependently from ~55 to 26 (P=0.001, L-NAMe=D-NAMe) (Figure 2). Addition of NP led to a return of dialysate pyruvate concentration and lactate/pyruvate ratio back to baseline levels.

**Norepinephrine**

To test if Ang II is able to facilitate sympathetic neurotransmission, adipose tissue and muscle were perfused with increasing doses of either Ang II or isoproterenol. In adipose tissue, dialysate norepinephrine concentration increased from 0.33±0.08 (baseline) to 0.52±0.04 nmol/L (P<0.05) during perfusion with 0.01 μmol/L Ang II (Figure 3). Higher Ang II concentration did not lead to a further increase in dialysate norepinephrine concentration. Perfusion with isoproterenol induced a dose-dependent increase in dialysate norepinephrine concentration from 0.42±0.04 (baseline) to 1.74±0.30 nmol/L at 1 μmol/L. In muscle, dialysate norepinephrine concentration did not change at any Ang II concentration used (Figure 3). However, during perfusion with isoproterenol, dialysate norepinephrine concentration increased from 0.84±0.14 (baseline) to 2.09±0.16 at 1 μmol/L.

**Discussion**

The main finding in our study is that interstitially applied Ang II elicits hemodynamic and metabolic responses that are not predicted from intravascular studies. Ang II is a strong vasoconstrictor when applied to the intravascular space. In contrast, we observed that Ang II has a minimal effect on the perfusion of adipose tissue and of skeletal muscle when applied to the interstitial space. The absence of an important hemodynamic effect of Ang II in both tissues may indicate that interstitial Ang II is less important for blood flow regulation than intravascular Ang II, at least in skeletal muscle and in abdominal adipose tissue. We used the ethanol dilution technique, which is based on the Fick principle, to assess changes in tissue blood flow. Using this methodology, we have previously shown that in skeletal muscle, interstitial epinephrine elicits a dose related decrease in tissue blood flow. In contrast, in abdominal adipose tissue epinephrine and phenylephrine did not decrease blood flow when applied through the microdialysis probe. Obviously, resting adipose tissue is already at its minimum level, at least in men. Nitroprusside and isoproterenol elicited a marked increase in skeletal muscle and in adipose tissue blood flow. Moreover, we were able to show a subtle interaction between adrenergic stimulation and NO. Thus, absence of a change in ethanol ratio with Ang II is indeed consistent with absence of a major change in blood flow.

Even though interstitial Ang II did not have a major effect on tissue blood flow, it elicited marked metabolic changes in two functionally different tissues, namely white adipose tissue and skeletal muscle. Ang II can bind to plasma membrane receptors on adipocytes but also on presynaptic fibers of sympathetic neurons enhancing transmitter release during nerve stimulation. Therefore, Ang II could influence lipid and carbohydrate metabolism directly through an effect on receptors on adipocytes and indirectly through activation of receptors on presynaptic fibers of adrenergic neurons. A combination of both mechanisms could also occur. In our study, Ang II application did not lead to a major change in interstitial norepinephrine concentrations. In contrast, stimulation of presynaptic β-2 adrenoreceptors increases interstitial norepinephrine markedly. However, the sensitivity of our method may not be sufficient enough to exclude a subtle effect of interstitial Ang II on norepinephrine turnover.

As indicated by the changes in dialysate glycerol concentrations, Ang II affected lipolysis in a tissue-specific manner: We observed a stimulation in adipose tissue but an inhibition in muscle, both dose-dependently. However, the increase in lipolysis with Ang II (+30%) was much weaker than the increase observed with isoproterenol (+300%). After binding of Ang II to its receptors, mainly Ang II type I (AT-1) receptors, various signal transduction systems are triggered, leading to an activation of phospholipases C, A2, and D, inhibition of adenylate cyclase, opening of calcium channels, or activation of tyrosine kinases. Inhibition of adenylate cyclase results in an inhibition of lipolysis and that could account for the antilipolytic effect of Ang II in muscle. Jones et al described a lipogenic effect of Ang II in differentiated murine 3T3-L1 preadipocytes and human adipocytes, along with increased expression and activity of the lipogenic enzymes glycerol-3-phosphate dehydrogenase and fatty acid synthase. The lipogenic effect of Ang II found in adipocytes in vitro contrasts with the small lipolytic effect
found in adipose tissue in vivo. It is unclear, however, whether the lipolytic effect in vivo is the consequence of the small but significant increase in dialysate norepinephrine during perfusion with Ang II or whether it is the result of some unspecific effects.

Blocking of NOS with L-NAME did not significantly modulate the Ang II–induced effects. However, as has been demonstrated recently, the L-NAME concentration used is effective for blocking NOS.Obviously, endogenously produced NO is not sufficient to interact with Ang II. In contrast, the NO donor NP attenuated the Ang II–induced effects on lipolysis. However, the NP concentration used is in the millimolar range and is effective to achieve maximum vasodilation. Therefore, the NP-induced effects can mostly be attributed to the increased tissue perfusion. In adipose tissue, dialysate glycerol decreased below baseline values. In skeletal muscle, dialysate glycerol increased close to baseline values. The glycerol decrease in adipose tissue is in line with the increase in blood flow that we observed and may indicate increased glycerol clearance. However, the glycerol increase in muscle close to baseline levels despite an increased blood flow indicates that NO activates basal lipolysis and counteracts the antilipolytic effect of Ang II. Gaudiot et al reported that NO exogenously added to isolated rat adipocytes stimulates basal lipolysis but inhibits stimulated lipolysis through cGMP-independent mechanisms that are tightly linked to the redox state of NO. Dialysate glycerol concentrations in adipose tissue tended to be increased with NOS inhibition. The lipolytic effect of NOS inhibition in adipose tissue in vivo is in contrast to the antilipolytic effect observed in adipocytes in vitro. However, NO is a tonic inhibitor of sympathetic nerve traffic, and inhibition of NOS is accompanied by an increase in both basal and stimulated lipolysis in adipose tissue in vivo, as has been shown recently.

In addition to the effects on lipolysis, Ang II markedly changed carbohydrate metabolism in skeletal muscle. Dialysate lactate did not change during perfusion with Ang II, whereas dialysate pyruvate increased and lactate/pyruvate ratio decreased dose-dependently. A decrease in lactate/pyruvate ratio is consistent with an increase in aerobic glycolysis. On the other hand, the changes in dialysate lactate, pyruvate, and lactate/pyruvate ratio are consistent with a hypoglycemic situation: The lower availability of glucose is compensated by a greater efficiency of glucose metabolism. Muscle blood flow and glucose supply were not significantly affected by Ang II. Obviously, Ang II moderately impaired glucose uptake into the muscle, leading to a metabolic response similar to a hypoglycemic situation. This interpretation is in line with a recent report by Frossard et al, who found an improved glucose uptake into muscle after treatment of volunteers with inhibitors of either ACE or Ang II receptors.

The Ang II doses used in our study may result in higher tissue levels of Ang II than will normally be encountered in vivo. Maximum perfusate concentration of Ang II used in our study was ≈1 × 10^–8 mol/L, whereas normal plasma concentration is ≈10 × 10^–12 mol/L. However, local generation of Ang II may result in higher Ang II concentrations in the interstitium than encountered in the circulation. In fact, interstitial concentrations of Ang II in rat kidney are much higher than plasma concentrations. Furthermore, in vitro recovery of Ang II is ≈20% at a flow rate of 2 μL/min when using CMA/60 catheters. Recovery could be even lower in vivo. We did not test for that. It is possible, however, that 10% of Ang II of the perfusate diffuses into the interstitial space. Taking this into account, a perfusate concentration of

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Changes in dialysate pyruvate concentration and in lactate/pyruvate ratio. Skeletal muscle was perfused as described in Figure 1. Data are given as mean±SEM, n=8; Ang II induced a dose-dependent increase in dialysate pyruvate and a decrease in lactate/pyruvate ratio. There was no impact of NOS inhibition by L-NAME, but the effects could be reversed by NP. *P<0.05 when compared with baseline for D- and L-NAME, respectively.

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** Changes in dialysate norepinephrine concentrations. Abdominal subcutaneous adipose tissue and skeletal muscle were perfused with Ringer’s solution + EtOH and increasing doses of either Ang II or isoproterenol (IP). Data are given as mean±SEM, n=4; Ang II induced a small but significant, dose-independent increase in dialysate norepinephrine. No changes were observed in muscle. IP elicited a marked increase in dialysate norepinephrine in both tissues at 1 μmol/L. *P<0.05 when compared with baseline for IP and Ang II, respectively.
0.1 μmol/L Ang II would correspond to an interstitial concentration of ≈0.01 μmol/L, a value that is already within the physiological range. Thus, although we cannot completely rule out that some of the effects observed at the higher Ang II doses in our study represent the pharmacological rather than the physiological action of Ang II, the magnitude of the Ang II effect probably corresponds to the physiological response of both tissues to Ang II.

In conclusion, interstitially applied Ang II elicits rather weak hemodynamic and metabolic effects in adipose tissue, whereas it dose-dependently inhibits lipolysis and glucose uptake in muscle, independent of its hemodynamic effects. The metabolic effects are not modulated by endogenously produced NO. However, most of the effects are reversible when NO-donor NP is added to the perfusate.

Perspectives
Our data suggest a tissue-specific effect of Ang II regarding the regulation of lipolysis and glucose metabolism. Ang II has no major effect on tissue perfusion in adipose tissue and muscle when applied interstitially. However, lipolysis is weakly stimulated in adipose tissue but strongly inhibited in muscle by Ang II. Furthermore, Ang II stimulates pyruvate production, whereas lactate production is unchanged, resulting in an increase in lactate/pyruvate ratio in muscle. This observation indicates an impaired glucose supply into muscle, possibly caused by a partial inhibited glucose uptake. Possibly, this effect is even more pronounced in obesity and can, therefore, promote the development of obesity-associated insulin resistance. All these effects are probably mainly but not exclusively related to activation of Ang II receptors on adipocytes or muscle cells/fibers rather than changes in norepinephrine release through activation of Ang II receptors on adipocytes or muscle cells.

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