

Distribution of angiotensin II receptors in rat and human adipocytes

David L. Crandall,^{1,*,**} Helen E. Herzlinger,^{**} Brian D. Saunders,^{**}
Douglas C. Armellino,^{**} and John G. Kral[†]

Departments of Medicine* and Surgery,[†] SUNY Health Science Center, Brooklyn, NY, and
Cardiovascular Research,^{**} Medical Research Division, American Cyanamid Company, Pearl River, NY

Abstract Angiotensin II (AII) receptor binding assays were performed in rat adipocytes from three separate anatomic depots. Fat cells were isolated by collagenase digestion, and plasma membranes were prepared from the epididymal, mesenteric, and retroperitoneal fat depots of male Sprague-Dawley rats at 100 days of age. Binding of ¹²⁵I-labeled [Sar¹,Ile⁸]AII was rapid, saturable, and specific in membranes from all depots, identifying a receptor with a similar affinity of approximately 1 nM. Site-associated differences in receptor number were observed, with epididymal and mesenteric fat cell membranes exhibiting significantly more receptors than retroperitoneal fat cells when binding was expressed per unit of membrane protein. When corrected for cell volume, the number of receptors per cell ranked epididymal > retroperitoneal > mesenteric. Inhibitory constants for the peptide agonists AII and AIII and the peptide antagonist [Sar¹,Ala⁸]AII indicated similar affinities in all three depots. Because the receptor has been classified pharmacologically into two subtypes, the AT₁ selective antagonist losartan, and the AT₂ selective antagonist PD 123,319 were used to classify the adipocyte receptor, indicating an AT₁ subtype with an affinity for losartan in the mesenteric and retroperitoneal adipocytes that was significantly greater than the epididymal. Similar studies were performed in adipocyte membranes obtained from human omental and subcutaneous adipose tissue, revealing the presence of an AII receptor in both depots with an affinity of approximately 10 nM for losartan. **Key words:** These data indicate site-specific differences in AII receptor number in fat cell membranes from rats and the existence of human adipocyte AII receptors, suggesting that the adipocyte is significant for the peripheral metabolism of components of the renin-angiotensin system.—Crandall, D. L., H. E. Herzlinger, B. D. Saunders, D. C. Armellino, and J. G. Kral. Distribution of angiotensin II receptors in rat and human adipocytes. *J. Lipid Res.* 1994. 35: 1378–1385.

Supplementary key words angiotensin II • receptor binding

Angiotensin II is a well-characterized peptide, which for decades was viewed only as a potent vasoconstrictor with profound effects on blood pressure and electrolyte balance (1, 2). The classic concept of control of angiotensin II (AII) metabolism via renin release from the kidney has more recently been revised to include peripheral tissue sites (3). Of the numerous tissues examined for com-

ponents of this “peripheral” renin-angiotensin system, adipose tissue has unexpectedly been implicated as the largest primary source of the precursor molecule, angiotensinogen (4). While adipocyte angiotensinogen production has also been shown to be tissue-specific, modulated by the nutritional state of the animal without effects on liver or serum concentrations of the hormone (4), the precise role of AII in adipose tissue remains unknown.

Several recent investigations have suggested a role for AII receptors in development, but these studies have been primarily performed in cultured cells or fetal tissues. Chronologic data generated from autoradiograms in late gestation fetus have indicated significant changes in AII receptor concentration with the stage of development (5), and expression of AII receptor subtypes have been observed to vary depending on the age of cultured fetal fibroblasts (6). A correlation between receptor subtype expression with the stage of development of rat brain has also been described (7), but as with other developmental studies, the in vivo applicability of these findings is unclear. Interestingly, we have recently identified an AII receptor in rat adipocyte membranes obtained from a single anatomic site in mature rats (8). Because adipose tissue is located in anatomically distinct depots with associated differences in specific physiological responses, the present study has investigated the distribution of AII receptors in both rat and human adipocyte membranes.

METHODS

Rat adipocyte membrane preparation

The experimental design initially involved determining the AII binding characteristics in adipocyte membranes

Abbreviations: AII, angiotensin II; ACE, angiotensin-converting enzyme.

[†]To whom correspondence should be addressed.

harvested from three different anatomic sites in approximately 100-day-old, male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA). The rats were maintained in a controlled environment with ad libitum food (Ralston Purina Rat Chow #5001, St. Louis, MO) and water. Rats were killed in the fed state on the morning of the experiment by carbon dioxide inhalation, and adipose tissue from the epididymal, mesenteric, and retroperitoneal depots was quickly excised and placed in cold saline. The spermatic artery and vein were removed from the epididymal fat, and brown fat deposits were dissected away from the retroperitoneal depot. The adipose tissue was then weighed and the process of adipocyte isolation was begun essentially using the method of Rodbell (9). Fat was minced into small pieces, and approximately 10 g each was transferred to a flask containing 25 ml of Krebs-Ringer-bicarbonate buffer (KRB), 6 mM glucose, and 50 mg of collagenase, pH 7.4. The flask containing the minced adipose tissue was shaken vigorously (150 strokes/min) at 37°C for 30 min, followed by passing of the contents through 150 μ m nylon mesh. Adipocytes passed freely into waiting tubes, while the undigested stromal-vascular tissue that was trapped on the screen was immediately transferred to cold Tris buffer (10 mM Tris-HCl, pH 8.0). The tubes containing the adipocytes were washed an additional 4 \times with 20 ml each of cold Tris buffer by first allowing the adipocytes to separate from the digestion medium by flotation, removing the infranatant through polyethylene tubing attached to a syringe, resuspending the cells in the Tris buffer, and repeating this procedure. Immediately after the final wash, an appropriate aliquot was taken for determination of the purity of the adipocyte preparation by microscopic inspection, and for morphologic analysis of cell volume (10, 11).

Adipocyte membranes were prepared essentially according to the method of Mauriege et al. (12), with minor modifications described previously by us (8). Using a Brinkmann polytron with a small blunt probe, adipocytes were homogenized for 20 sec at a medium speed setting, and the resulting homogenate was centrifuged at 40,000 g for 35 min at 4°C. After centrifugation, the fat cake and supernatant were carefully removed and discarded, and the pellet was resuspended in 1 ml of Tris buffer. Protein content of the resuspended membrane was determined by the method of Lowry et al. (13), and binding assays were either performed immediately, or after storage at -75°C for no longer than 1 week. Stromal-vascular tissue was homogenized and centrifuged also, and aliquots of the resuspended pellet were used as a positive control for the detection of vascular cells using biochemical techniques.

Purity of the adipocyte preparation

In order to insure that other cell types from the adipose tissue were not being isolated with the adipocytes, several additional assays were performed. Angiotensin-converting

enzyme activity was determined (14) in the fat cell and stromal-vascular fractions by using a tritiated substrate for the enzyme, hippuric acid (Ventrex, Portland, ME). Immunohistochemistry was also performed on the adipocyte preparation to determine the potential contamination by endothelial cells from the vasculature (15). A rabbit anti-human polyclonal antibody for Von Willebrand factor (Factor VIII; Sigma Chemical, St. Louis, MO) was diluted 1:200 and then incubated with the adipocytes for 60 min at 37°C, followed by washing and incubation with the second antibody, a fluorescein-conjugated goat antibody to the F(ab')₂ fragment of rabbit IgG. Cultured bovine aortic endothelial cells served as the positive control. Finally, inspection of each adipocyte preparation was performed at 100 \times magnification prior to homogenization.

Rat adipocyte binding studies

Membranes were diluted with 0.25% BSA buffer (bovine serum albumin, 50 mM Tris-HCl, 5 mM MgCl₂, pH 7.4) to a final concentration of 5 μ g protein/10 μ l buffer. For Scatchard analysis, membranes were incubated with 12 different concentrations of ¹²⁵I-labeled [Sar¹,Ile⁸]AII (NEN, Boston, MA) ranging from 0.15 to 5.0 nM. A typical incubation tube contained 160 μ l (80 μ g) fat cell membrane protein, 20 μ l of isotope, 20 μ l of 1 μ M unlabeled AII for determination of nonspecific binding, or 20 μ l of buffer for the measurement of total binding. The antagonist to the receptor was used in order to prevent any G-protein linked effect associated with agonist binding. Kinetics of binding have been described in detail previously (8). The assay was initiated by the addition of the protein, followed by incubation at 22°C for 30 min in a slowly shaking water bath. Separation of bound from free radioactivity was performed on a Brandel harvesting apparatus containing a Whatman GF/B filter, followed by six additional rinses with 5 ml each of cold 0.9% saline. The filters were placed in tubes, and radioactivity was measured in a gamma scintillation counter programmed to correct for the half-life of the isotope (Packard Cobra 5010; 80% counting efficiency). Data were processed by RS232 interface directly into a VAX mainframe computer containing software for determination of equilibrium dissociation rates (K_D) and maximal binding sites (B_{max}) (Lundon, Inc., Chagrin Falls, OH).

The relative affinities of adipocyte membranes for the agonists AII and angiotensin III (AIII), the nonselective peptide antagonist [Sar¹,Ala⁸]AII, the selective AT₁ antagonist losartan (DuP 753), and the selective AT₂ antagonist PD 123,319 were also determined. (Losartan was a generous gift from Dr. Ronald Smith of DuPont Merck Pharmaceutical Company. PD 123,319 was a kind donation by Dr. Harvey Kaplan of Warner-Lambert Company.) Fat cell membranes were incubated in the presence of 1 nM ¹²⁵I-labeled [Sar¹,Ile⁸]AII together with varying concentrations of antagonists ranging from 10⁻⁶

to 10^{-13} M, and in increasing graduations of one-third log unit. Nonspecific binding was determined in the presence of $1 \mu\text{M}$ unlabeled AII. The concentration range for PD 123,319 was from 10^{-3} to 10^{-10} M. Relative inhibitory constants required to displace 50% of bound ligand (IC_{50}) were calculated using the Lundon-2 software program. Statistical analysis was performed using a two-way analysis of variance for multiple groups (Statistica/Mac software, StatSoft, Tulsa, OK). Group means were considered significantly different at $P < 0.05$.

Degradation of ^{125}I -labeled $[\text{Sar}^1, \text{Ile}^8]\text{AII}$ was determined using the method described for insulin degradation in adipocytes by Bolinder et al. (16). Briefly, adipocyte membranes were incubated in the presence of the radioligand as in the binding assays, then protein was precipitated with 15% (vol/vol) trichloroacetic acid. AII degradation was measured as soluble radioactivity and expressed as a percentage of total radioactivity added to the buffer.

Human adipocyte binding studies

Human adipose tissue samples were obtained from obese patients undergoing gastroplasty. Written informed consent was obtained from all patients and the protocol was approved by the Institutional Review Board. After an overnight fast and under general anesthesia, knife biopsies of 1–5 g of adipose tissue were taken immediately upon entering the abdomen through an upper midline incision. Samples were taken of the epigastric subcutaneous fat and the peripheral omentum. The tissue was placed in normal saline on ice for transporting to the laboratory, and processed within 1 h. Adipocyte membrane preparations from human adipose tissue were prepared as described for rat adipose tissue. Scatchard analysis and estimates of inhibitory constants for losartan and PD 123,319 were also determined. Estimates of number of receptor sites were determined from nonlinear transformations of displacement curves using the Lundon-2 software.

TABLE 1. AII receptor binding data from rat adipocyte membranes

	Adipose Tissue Depot		
	Epididymal	Mesenteric	Retroperitoneal
Depot weight (g)	4.48 (0.47)	4.47 (0.79)	5.24 (0.65)
Adipocyte volume (pl)	241 ^b (11)	161 ^{a,b} (22)	363 (18)
B_{max} (fmol/mg protein)	57.7 ^b (6.9)	42.9 ^b (5.6)	26.8 (5.4)
K_D (nM)	1.53 (0.20)	1.64 (0.30)	1.28 (0.22)

Values are mean (\pm SEM) from eight individual rats with mean body weight of 436 ± 10 g.

Significantly different from ^aepididymal or ^bretroperitoneal with value at $P < 0.01$.

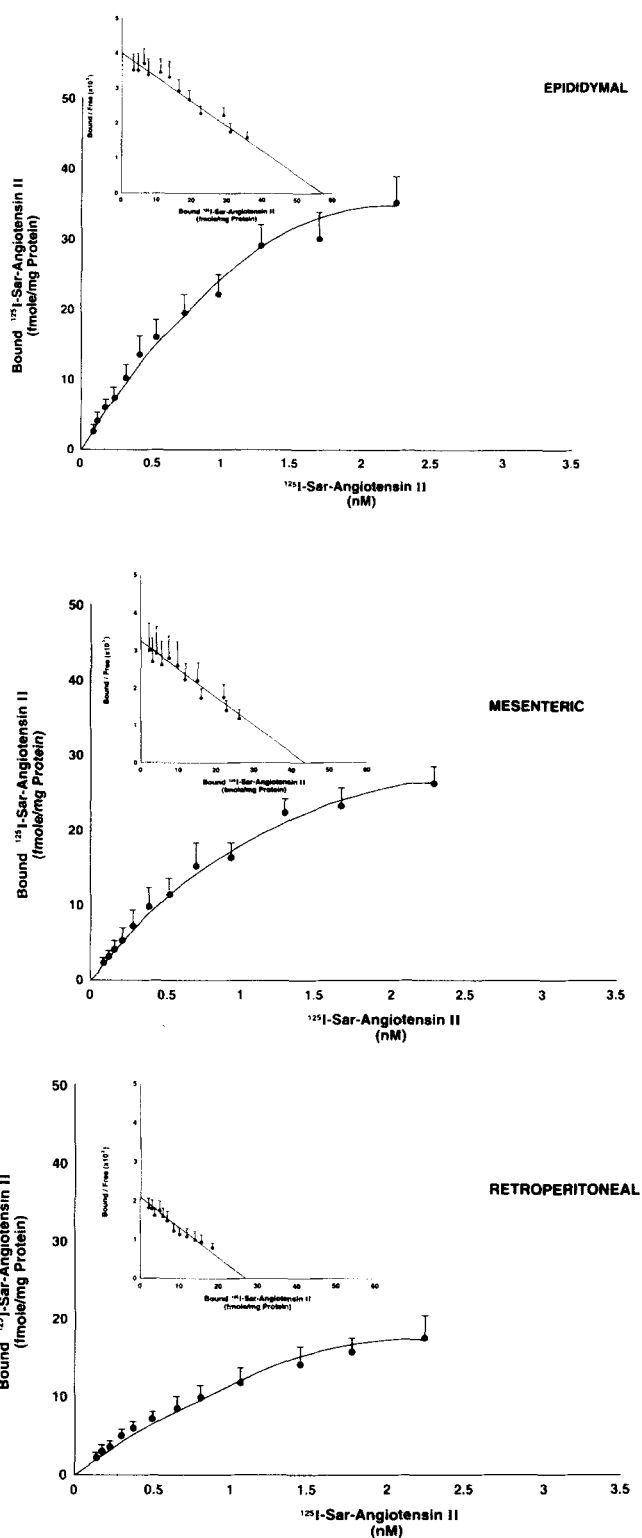


Fig. 1. Saturation of ^{125}I -labeled $[\text{Sar}^1, \text{Ile}^8]\text{angiotensin II}$ to adipocyte membranes from three anatomically distinct depots. Each point represents specific binding and is the mean of 6–7 separate experiments from individual rats. Scatchard analysis of these data is shown in the inserts. The negative reciprocal of the slope is equal to the K_D and the intercept with the abscissa indicates the maximum binding capacity, details of which are included in Table 1.

RESULTS

The binding data for rat adipocyte membranes using Scatchard analysis are shown in **Table 1** and **Fig. 1**. Epididymal, mesenteric, and retroperitoneal membranes bound the ligand in a saturable manner, with equivalent affinities of approximately 1 nM. There was a significant difference in B_{max} between anatomic sites when binding was expressed per mg protein, however, with the retroperitoneal depot exhibiting fewer binding sites. Importantly, because each depot contained adipocytes of varying volume, differences observed in binding were subsequently corrected for cell size. As shown in **Fig. 2**, when expressed as receptors per cell, there was a statistically significant difference in the number of sites per cell in the rank order of epididymal > retroperitoneal > mesenteric.

The IC_{50} s for a variety of peptide and nonpeptide agonists and antagonists are shown in **Table 2** and **Fig. 3**. These data indicate that the adipocyte membranes from a variety of anatomic sites exhibit a high affinity AII receptor that readily binds agonists and antagonists. Binding affinities for the peptide agonists AII and AIII and the peptide antagonist [Sar¹,Ala⁸]AII were similar in all three depots. Characterization of the subtype of the receptor using selective organic antagonists indicated a high affinity for the AT₁ subtype antagonist losartan, and a low affinity for the AT₂ subtype antagonist PD 123,319. While these data indicate that the adipocyte receptor is predominantly of the AT₁ subtype, it is interesting to note that the affinity of the receptor for losartan was significantly greater in the retroperitoneal and mesenteric than the epididymal depot. Therefore, those adipocytes with the fewest number of binding sites per cell exhibited the higher affinity for losartan.

The integrity of radioligand and the purity of the cellular composition of the membrane preparation used for

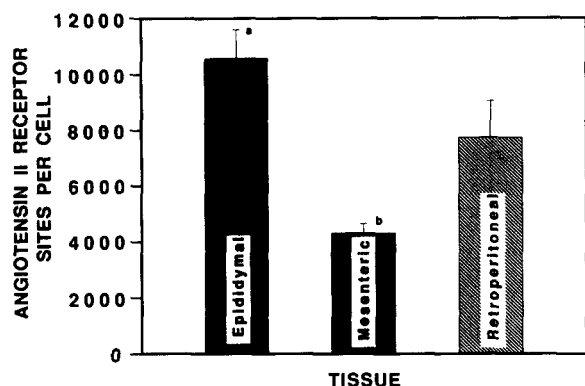


Fig. 2. Analysis of AII receptor binding in adipocytes with data corrected for cell volume and expressed as sites per cell. ^aSignificantly different from mesenteric at $P < 0.01$ and retroperitoneal at $P < 0.05$. ^bSignificantly different from retroperitoneal at $P < 0.05$.

TABLE 2. Relative inhibitory constants (IC_{50}) of specific compounds for binding to rat adipocyte AII receptor

Compound	Adipose Tissue Site		
	Epididymal	Mesenteric	Retroperitoneal
AII	3.34×10^{-9} (0.25)	3.93×10^{-9} (0.31)	3.71×10^{-9} (0.45)
AIII	2.23×10^{-8} (0.69)	1.75×10^{-8} (0.20)	1.85×10^{-8} (0.43)
Sar ¹ Ala ⁸ AII	4.32×10^{-9} (0.35)	3.90×10^{-9} (0.23)	2.63×10^{-9} (0.26)
Losartan (AT ₁)	1.30×10^{-8} (0.23)	0.68×10^{-8} ^a (0.09)	0.85×10^{-8} ^a (0.18)
PD 123,319 (AT ₂)	1.73×10^{-4} (0.23)	2.02×10^{-4} (0.54)	2.42×10^{-4} (0.36)

Values are mean with SEM in parentheses of $n = 3-7$ individual animals for each compound; units = M. Full dose range displacement of binding is shown in Fig. 3.

^aSignificantly different from affinity for losartan in epididymal depot at $P < 0.05$.

binding were also determined. The binding of radioligand was similar in adipocyte membrane preparations isolated in either a buffer containing a combination of the protease inhibitors leupeptin (5 μ g/ml), soybean trypsin inhibitor (25 μ g/ml), bacitracin (140 μ g/ml), pepstatin A (7 μ g/ml), and phenylmethylsulfonyl fluoride (PMSF) (100 μ M) or with exhaustive rinsing and homogenization in Tris buffer alone as described above in Methods. Degradation experiments indicated no significant degradation by the adipocyte membrane preparation under the incubation conditions used for binding at 22°C in either rat or human fat cells, with soluble fraction radioactivity after protein degradation essentially equivalent to nonspecific binding. To insure that the binding was not influenced by the presence of cell types other than adipocytes, a biochemical assay for angiotensin-converting enzyme (ACE), an enzyme of stromal-vascular origin, and a immunohistochemical assay for von Willebrand factor, an endothelial cell marker, were performed (15). ACE activity ranged from 59 ± 10.4 units/mg protein in the stromal-vascular fraction of the retroperitoneal adipose tissue to 202.1 ± 31.9 units/mg protein in the stromal-vascular fraction of the epididymal depot. At no time was ACE activity observed in the adipocyte fraction of any depot, even though all analyses were performed in homogenates of at least 20 million fat cells, a number of cells equal to or greater than that used for membrane preparation. Immunohistochemical analysis indicated the presence of von Willebrand factor in cultured bovine endothelial cells, but no positive fluorescence was observed in the adipocyte preparations of each depot (**Fig. 4**). Finally, visual inspection of the adipocyte preparations revealed no contamination by other cell types.

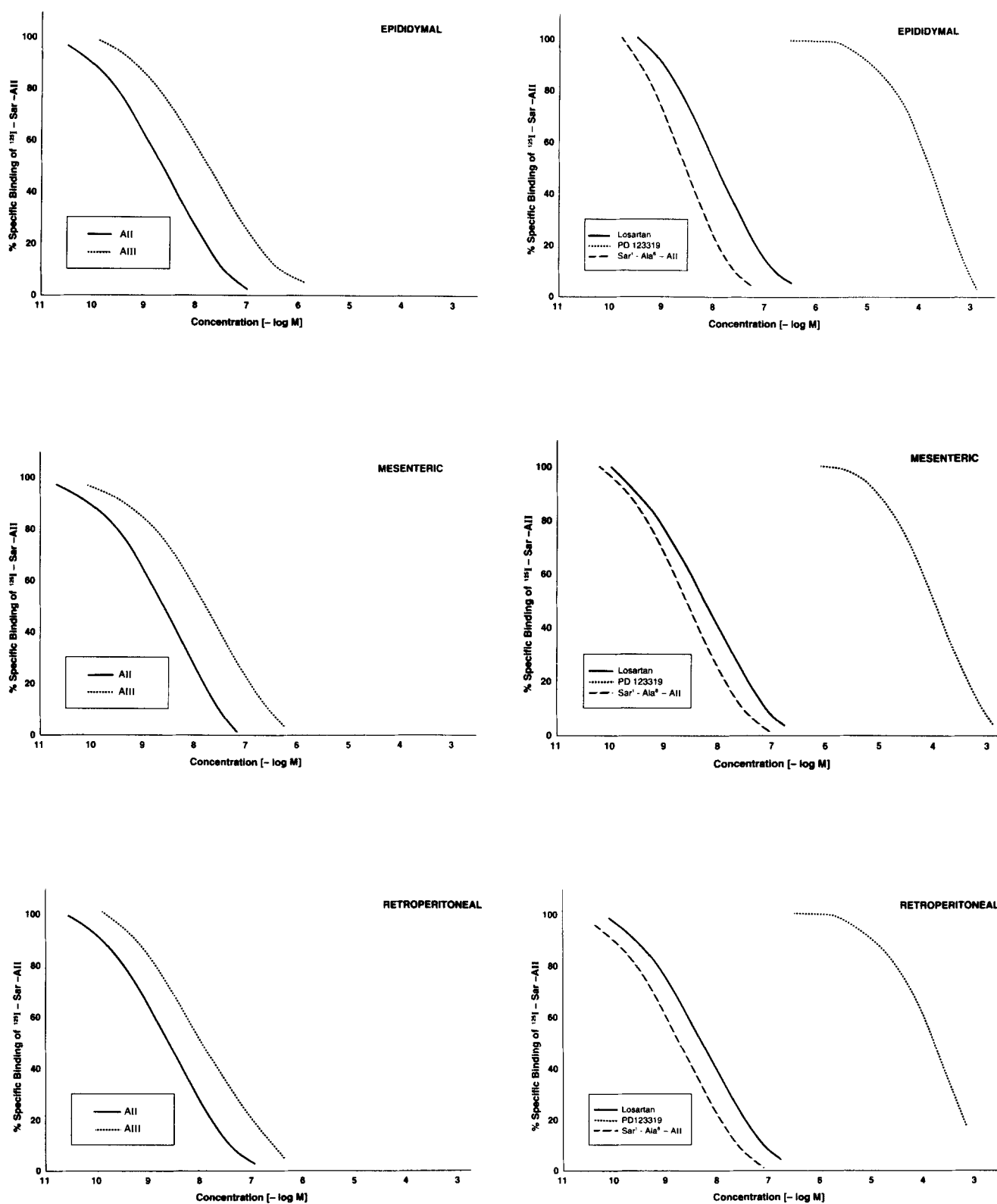


Fig. 3. Displacement of ^{125}I -labeled $[\text{Sar}^1, \text{Ile}^8]$ angiotensin II bound to fat cell membranes isolated from three different anatomic locations. Agonists (left) and antagonists (right) for the receptor are shown in separate panels. Each curve represents the best fit of 12 separate competitor concentrations, and mean data points have not been included for clarity. Mean IC_{50} values \pm SEM are provided for each compound in Table 2.

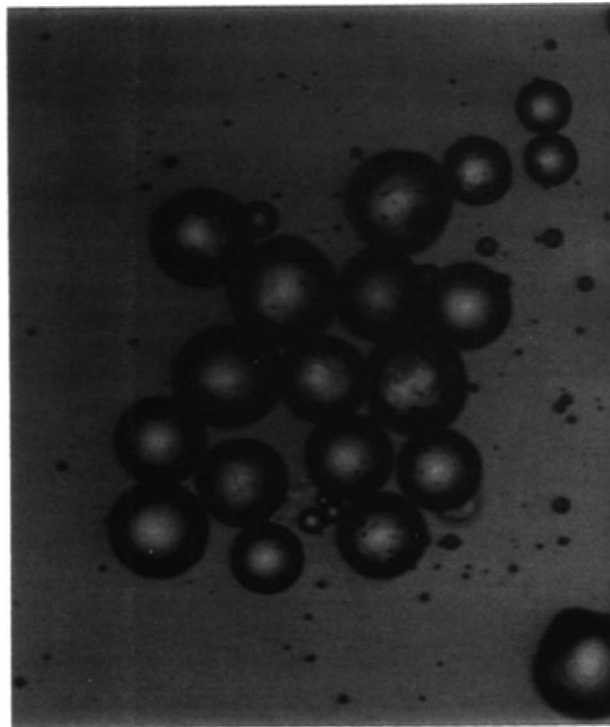
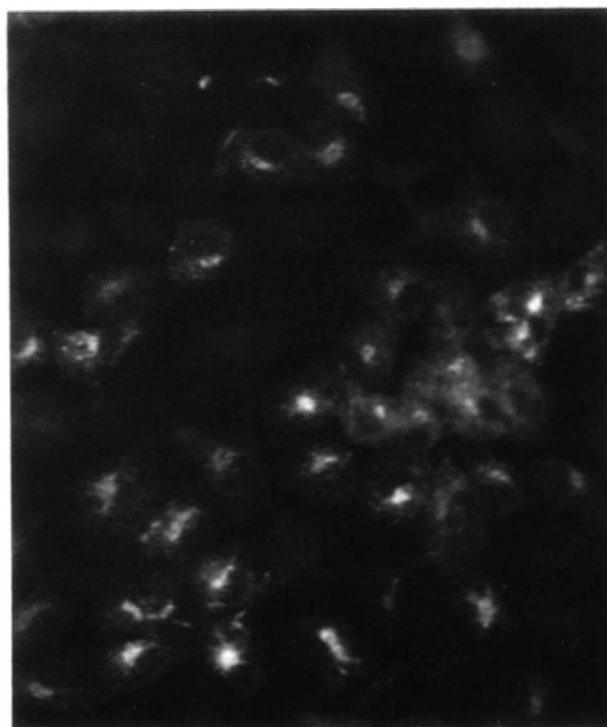


Fig. 4. Immunohistochemical identification of von Willebrand factor in endothelial cells. Granules of the factor are clearly identified intracellularly in cultured bovine endothelial cells (left panel), which is absent from the adipocyte preparation (right panel). Magnification of each panel is 195 \times .

Binding data for human adipose tissue are shown in **Table 3** and **Fig. 5**. Adipocyte membranes harvested from either the omental or subcutaneous adipose tissue of obese individuals were used for Scatchard analysis and estimates of IC_{50} s. Because of limited amounts of tissue, only the subtype specific antagonists losartan and PD 123,319 were used for IC_{50} determinations. Human adipocyte membranes exhibited an affinity for the AT_1 subtype specific antagonist losartan similar to that observed in rat, with no significant inhibition by the AT_2 subtype antagonist PD 123,319 (data not shown).

TABLE 3. Values for AII receptor binding in human adipocytes

Tissue Site	B_{max} (fmol/mg protein)	IC_{50} Losartan (M)
Omental	31.2 (10.2)	0.93×10^{-8} (0.34)
Subcutaneous	27.3 (9.3)	1.61×10^{-8} (0.45)

Values are mean (\pm SEM) of four patients undergoing gastroplasty with average BMI of 51 (range 38–59). Typical binding experiment is shown in Fig. 5.

DISCUSSION

In addition to the well-characterized pharmacologic actions of AII on blood pressure, several laboratories have observed variations in AII receptor binding in developing tissues, suggesting an additional role for this peptide (7, 17, 18). While the majority of these studies have been performed in brain, some additional experiments have been described in cultured fibroblasts "aged" by repetitive passaging, as well as in situ hybridization to a variety of rat fetal organs (5, 6). The general focus of these studies has been the characterization of AII receptor subtypes during different stages of tissue development. To date, the possible role of angiotensin II in growth remains largely unresolved, and experimentation has been predominantly limited to cell culture assays or membrane binding in tissues with limited growth potential.

Adipose tissue mass can continue to increase throughout most of adult life by a combination of adipocyte hypertrophy and hyperplasia, and the pattern of cellular growth of the fat depot is dependent upon its anatomic location in both rodents and humans (19–21). Because of the potential effect of AII upon growth, we recently investigated AII binding in rat fat cells, and for the first time identified a high affinity receptor for AII in adipocytes

HUMAN SUBCUTANEOUS ADIPOCYTES

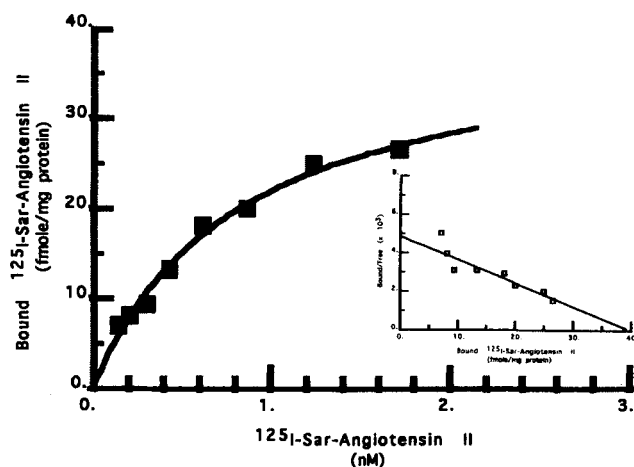


Fig. 5. Typical saturation of ^{125}I -labeled $[\text{Sar}^1, \text{Ile}^8]$ angiotensin II to human subcutaneous adipocyte membranes. Each point represents specific binding of the ligand. Scatchard analysis of the data is shown in the insert.

from a single depot in mature rats (8). We have presently expanded these initial findings by characterizing the AII receptor in several other depots of rats and in obese humans. These experiments demonstrate that rat adipocytes express the AII receptor regardless of depot location, indicate that fat cells predominantly exhibit the AT_1 receptor subtype, yet reveal significant differences in the numbers of receptors between depots. When corrected for cell volume, there are significant differences in the number of receptors per cell. Our studies, therefore, differ from those cited earlier describing AII receptors in cell culture systems (6) or brain (7), in which development was hypothesized to be regulated through AT_1 and AT_2 subtype expression. Conversely, we have observed a single receptor subtype in adipocytes, the expression of which varies among depots. Interestingly, in those cells with the fewest numbers of receptors, mesenteric and retroperitoneal adipocytes, a significantly higher affinity for losartan was observed. While the meaning of these data is presently unclear, it is important to note that isoforms of the AT_1 receptor, AT_{1A} and AT_{1B} , have been described using molecular techniques (22). Future experiments using molecular probes for each receptor isoform might aid in interpretation of these data. If different isoforms of the AT_1 receptor do in fact exist on adipocytes from different anatomic sites, organic antagonists with site-selective inhibitory properties would provide insight into the function of the AII receptors observed in these experiments, including their potential involvement in adipose tissue development.

Studies in human adipocytes from obese patients indicated an AII receptor similar in concentration and affinity

to that observed in rat, clearly establishing the expression of this receptor by human fat cells. Exogenous AII added to rat and human adipocytes in vitro results in an elevation of prostaglandins (PG) in the medium (23), indirectly indicating the only proposed function of the receptor, PG release. One of the prostaglandins released, PGE_2 , is a potent antilipolytic agent (24), and inhibition of its local production by blockade of the fat cell AII receptor would elevate basal lipolysis, potentially affecting adipocyte size and metabolism. This initial hypothesis needs to be re-evaluated, however, in the context of new data identifying components of the adipose tissue renin-angiotensin system, including the fat cell AII receptor (8), high concentrations of ACE in rat and human adipose tissue (14, 25), and significant amounts of adipocyte angiotensinogen, the synthesis of which is regulated nutritionally (4). As infusion of exogenous AII into dog subcutaneous adipose tissue inhibits glucose uptake and decreases fatty acid outflow following sympathetic stimulation (26), local production of AII by adipocytes could have similar effects on glucose metabolism. This hypothesis is supported by the establishment of components of the renin-angiotensin system in adipose tissue, and indirectly by clinical data indicating that angiotensin-converting enzyme (ACE) inhibitors have both anecdotally (27), and in controlled clinical trials (28), been associated with hypoglycemia in insulin-dependent and non-insulin-dependent diabetic patients. ACE inhibitors have also been shown to improve insulin sensitivity in hypertensive patients (29). A hypoglycemic response is not always associated with administration of ACE inhibitors to diabetic hypertensives, however, probably due in part to the multiple etiologies and combination therapies used to treat both hypertension and diabetes (30). It is important to note that the effect of ACE inhibitors on adipose tissue metabolism has not been specifically studied either in the laboratory or clinical setting, and no data are available on these agents in patients with metabolic disorders such as NIDDM without underlying hypertension, even though significant populations of normotensive obese patients exhibiting this clinical profile exist.

Administration of inhibitors of the renin-angiotensin system has also been associated with body weight reduction in animal (31–33) and human (34) studies, but again, the experimental design focused on hemodynamic and metabolic effects in tissues other than adipose tissue, and caloric intake was not monitored. Currently, new drugs are being developed for AII receptor blockade and renin enzyme inhibition for the treatment of hypertension, indicating that agents capable of inhibiting multiple sites of the renin-angiotensin system with distinct clinical profiles will be available in the near future. While the role of AII in adipose tissue physiology is yet to be completely determined, it has established effects on blood flow and metabolism, and may directly or indirectly effect growth

of adipose tissue. The identification of high affinity receptors for AII in human adipocyte membranes and the widespread use of therapeutic agents that affect either the synthesis of AII or blockade of its membrane receptor suggest that additional experimentation is warranted. ■

We appreciate the assistance of Dr. Roger French, Department of Statistics and Data Management, in the analysis of the data, and Dr. Gary Hausman for immunohistochemical techniques.

Manuscript received 22 September 1993 and in revised form 18 February 1994.

REFERENCES

- Haber, E., J. Sancho, S. Burton, and A. C. Barger. 1975. The role of the renin-angiotensin-aldosterone system in cardiovascular homeostasis in normal man. *Clin. Sci. Mol. Med.* **48**: 49s-52s5.
- Peach, M. J. 1977. Renin-angiotensin system: biochemistry and mechanisms of action. *Physiol. Rev.* **57**: 313-370.
- Campbell, D. J. 1987. Circulating and tissue angiotensin systems. *J. Clin. Invest.* **79**: 1-6.
- Frederich, R. C., B. B. Kahn, M. J. Peach, and J. S. Flier. 1992. Tissue-specific nutritional regulation of angiotensinogen in adipose tissue. *Hypertension.* **19**: 339-344.
- Millan, M. A., P. Carvallo, S. I. Izumi, S. Zemel, K. Catt, and G. Aguilera. 1989. Novel sites of expression of functional angiotensin II receptors in the late gestation fetus. *Science.* **244**: 1340-1342.
- Johnson, M. C., and G. Aguilera. 1991. Angiotensin-II receptor subtypes and coupling to signaling systems in cultured fetal fibroblasts. *Endocrinology.* **129**: 1266-1274.
- Tsutsumi, K., and J. M. Saavedra. 1991. Characterization and development of angiotensin II receptor subtypes (AT₁ and AT₂) in rat brain. *Am. J. Physiol.* **26**: R209-R216.
- Crandall, D. L., H. E. Herzlinger, B. D. Saunders, R. C. Zolotor, L. Feliciano, and P. Cervoni. 1993. Identification and characterization of angiotensin II receptors in rat epididymal adipocyte membranes. *Metabolism.* **42**: 511-515.
- Rodbell, M. 1964. Metabolism of isolated fat cells. *J. Biol. Chem.* **239**: 375-380.
- Crandall, D. L., B. Goldstein, F. Huggins, and P. Cervoni. 1984. Adipocyte blood flow: influence of age, anatomic location, and dietary manipulation. *Am. J. Physiol.* **247**: R46-R51.
- DiGirolamo, M., S. Mendlinger, and J. W. Fertig. 1971. A simple method to determine fat cell size and number in four mammalian species. *Am. J. Physiol.* **221**: 850-858.
- Mauriege, P., J. Galitzky, M. Berlan, and M. Lafontan. 1987. Heterogeneous distribution of beta and alpha₂ adrenoceptor binding sites in human fat cells from various fat depots: functional consequences. *Eur. J. Clin. Invest.* **17**: 156-165.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
- Crandall, D. L., G. Gordon, H. E. Herzlinger, B. D. Saunders, R. C. Zolotor, P. Cervoni, and J. G. Kral. 1992. Transforming growth factor alpha and atrial natriuretic peptide in white adipose tissue depots in rats. *Eur. J. Clin. Invest.* **22**: 676-680.
- Kern, P. A., A. Knedler, and R. H. Eckel. 1983. Isolation and culture of microvascular endothelium from human adipose tissue. *J. Clin. Invest.* **71**: 1822-1829.
- Bolinder, J., P. Engfeldt, J. Östman, and P. Arner. 1983. Site differences in insulin receptor binding and insulin action in subcutaneous fat of obese females. *J. Clin. Endocrinol. Metab.* **57**: 455-460.
- Obermüller, N., T. Unger, J. Culman, P. Gohlke, M. de Gasparo, and S. Bottari. 1991. Distribution of angiotensin II receptor subtypes in rat brain nuclei. *Neurosci. Lett.* **132**: 11-15.
- Tsutsumi, K., and J. M. Saavedra. 1992. Heterogeneity of angiotensin II AT₂ receptors in the rat brain. *Mol. Pharmacol.* **41**: 290-297.
- Hirsch, J., and P. W. Han. 1969. Cellularity of rat adipose tissue: effects of growth, starvation, and obesity. *J. Lipid Res.* **10**: 77-82.
- Sjöström, L., U. Smith, P. Björntorp, and M. Krotkiewski. 1972. Cellularity in different regions of adipose tissue in young men and women. *Metabolism.* **21**: 1143-1153.
- Ohlson, L. O., B. Larsson, K. Svardsudd, L. Welin, H. Eriksson, L. Wilhelmsen, P. Björntorp, and G. Tibblin. 1985. The influence of body fat distribution on the incidence of diabetes mellitus: 13.5 years of follow-up of the participants in the study of men born in 1913. *Diabetes.* **34**: 1055-1058.
- Iwai, N., and T. Inagami. 1992. Identification of two subtypes in the rat type I angiotensin II receptor. *FEBS Lett.* **298**: 257-260.
- Richelsen, B. 1987. Factors regulating the production of prostaglandin E₂ and prostacyclin in rat and human adipocytes. *Biochem. J.* **247**: 389-394.
- Crandall, D. L., V. Vu, F. H. Lizzo, B. A. Davis, and P. Cervoni. 1987. Antihypertensive activity of viproston, a transdermally active antihypertensive PGE₂ analog. *Prostaglandins.* **33**: 767-773.
- Crandall, D. L., H. Herzlinger, P. Cervoni, T. Scalea, and J. G. Kral. 1991. Vasoactive substances in human adipose tissue biopsies. In *Obesity in Europe*. G. Ailhaud, B. Guy-Grand, M. Lafontan, and D. Ricquier, editors. John Libbey, London. 405-409.
- Belfrage, E., P. Hjendahl, and B. B. Fredholm. 1979. Metabolic effects of blood flow restriction in adipose tissue. *Acta Physiol. Scand.* **105**: 222-227.
- Bell, D. S. H. 1992. Hypoglycemia induced by enalapril in patient with insulin resistance and NIDDM. *Diabetes Care.* **15**: 934-936.
- Chan, J. C., C. S. Cockram, M. G. Nicholls, C. K. Cheung, and R. Swaminathan. 1992. Comparison of enalapril and nifedipine in treating non-insulin-dependent diabetes with hypertension: one year analysis. *Br. Med. J.* **305**: 981-985.
- Pollare, T., H. Lithell, and C. Berne. 1989. A comparison of the effects of hydrochlorothiazide and captopril on glucose and lipid metabolism in patients with hypertension. *N. Engl. J. Med.* **321**: 868-873.
- Moore, M. P., T. W. Elliott, and M. G. Nicholls. 1988. Hormonal and metabolic effects of enalapril treatment in hypertensive subjects with NIDDM. *Diabetes Care.* **11**: 397-401.
- Hashimoto, K., K. Imai, S. Yoshimura, and T. Ohtaki. 1981. Twelve month studies on the chronic toxicity of captopril in rats. *J. Toxicol. Sci.* **6(Suppl II)**: 215-246.
- Richer, C., M. Doussau, and J. F. Giudicelli. 1982. MK421 and prevention of genetic hypertension development in young spontaneously hypertensive rats. *Eur. J. Pharmacol.* **79**: 23-29.
- Morton, J., E. Beattie, and F. MacPherson. 1992. Angiotensin II receptor antagonist losartan has persistent effects on blood pressure in the young spontaneously hypertensive rat: lack of relation to vascular structure. *J. Vasc. Res.* **29**: 264-269.
- Enalapril Study Group. 1984. Enalapril in essential hypertension: a comparative study with propranolol. *Br. J. Clin. Pharmacol.* **18**: 51-56.