Short communication

Rat Epididymal Fat Tissue Express all Components of the Renin-angiotensin System

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Abstract. In the present study the gene expression of components of the renin-angiotensin system was investigated in fat tissue of rats. mRNAs for angiotensinogen, renin, angiotensin-converting enzyme and type I (AT_1) angiotensin II receptor were detected in the stromal-vascular fraction of the fat tissue and the same mRNAs, with the exception of the angiotesin-converting enzyme, in the adipocyte fraction. Renin and angiotensin-converting enzyme activity was measured. The main source of renin activity was found in adipocytes and some minor activity in the stromal-vascular fraction, while the majority of the angiotensin-converting enzyme activity was in the stromal-vascular fraction. The present data provide evidence for the presence of the active renin-angiotensin system in rat adipose tissue.

Key words: Renin — angiotensin system — Adipose tissue — Rat

Human adipose tissue is known to express all components of the renin-angiotensin system (RAS) with the exception of renin and AT_2 subtype of angiotensin II receptors (Engeli et al. 1999). Similarly, the rat adipose tissue expresses exclusively AT_1 receptors (Crandall et al. 1994; Zorad et al. 1995). In addition, angiotensinogen (AGT) mRNA, angiotensin-converting enzyme (ACE) and renin activities were observed in rat fat tissue (Crandall et al. 1992, Harp and DiGirolamo 1995). ACE activity is localised mainly in the stromal-vascular fraction of fat tissue (Crandall et al. 1992). However, complete expression of RAS components and their localisation in the rat fat tissue has not yet been known.

The purpose of this study was to investigate the gene expression of all components of RAS and to determine renin and ACE activities in isolated adipocytes and the stromal-vascular fraction of rat epididymal fat tissue.

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In our experiments, male specific pathogen free Sprague-Dawley rats (280-350 g, 3-month old; obtained from Charles River Laboratories; Sulzfeld, Germany) were used. The animals were decapitated and epididymal fat tissue was excised, rapidly frozen in liquid nitrogen and stored until assayed. Total RNA was isolated according to the method of Chomczynski and Sacchi (1987).

Five μg of total RNA was used for reverse transcription using Ready-To-Go You-Prime First-Strand Beads Kit (Amersham Pharmacia Biotech) and $pd(N)_6$ random hexamer primers (Pharmacia Biotech). Four μl of cDNA obtained was subsequently used in polymerase chain reaction (PCR) for amplification of fragments of the angiotensinogen, renin, ACE and AT_1 receptors. As negative controls, PCR from RNA was performed with reverse transcription omitted. Since no signal was obtained, we are convinced that the fragment obtained after RT-PCR was from the corresponding mRNA rather than coming from genomic contamination. Oligonucleotide sequences of the primers used in PCR, the optimal annealing temperature as well as the number of cycles are indicated in Table 1. PCR products were separated by electrophoresis in 2 % agarose gels in the presence of ethidium bromide stain.

Table 1. Oligonucleotide sequences of primers used in PCR for amplification of specific fragments of angiotensinogen, renin, ACE and AT1 receptor genes together with citation, optimal annealing temperatures and number of cycles in PCR.

Oligonucleotide sequences of primers	Annealing temperature and time	Number of cycles	PCR product (bp)	Reference
Angiotensinogen 5′- TTG TTG AGA GCT TGG GTC CCT TCA-3′ 5′- CAG ACA CTG AGG TGC TGT TGT CCA-3′	58 ℃/1′	35	263	Shyu et al. 1995
ACE 5′- CCT GAT CAA CAA GGA GTT TGC AGA G-3′ 5′- GCC AGC CTT CCC AGG CAA ACA GCA C-3′	58 ℃/1′	35	320	Iwai et al. 1995
Renin 5′- TCT CAG CAA CAT GGA CTA TGT GC-3′ 5′- TTA GCG GGC CAA GGC GAA CC-3′	56 ℃/1′	35	190	Pieruzzi et al. 1995
AT1 5′- GCA CAA TCG CCA TAA TTA TCC-3′ 5′- CAC CTA TGT AAG ATC GCT TC-3′	54 ℃/1′	30	444	Llorens-Cortes et al. 1994
Beta-actin 5′- AGT GTG ACG TTG ACA-3′ 5′- GAC TGA TCG TAC TCC TGC-3′	58 ℃/1′	35	240	Lou et al. 1995

Adipocytes and stromal-vascular fraction were isolated from epididymal fat tissue by collagenase digestion (Rodbel 1964; Crandall et al. 1992). The cytosol of both fractions was isolated as follows: isolated adipocytes and stromal-vascular fraction were homogenised by sonication in 20 mmol/l Tris buffer containing 0.3 mol/l sacharose and 1 mmol/l phenyl methyl sulfonylfluoride (PMSF). The homogenates were centrifuged at $600 \times g$ for 10 min at $4\,℃$. The liquid phase was transferred into new tubes and centrifuged at $11,000 \times g$ for 20 min at $4\,℃$. The supernatant was stored for further analysis at $-20\,℃$. Renin activity was determined in cytosol isolated from adipocytes and stromal-vascular fraction as the amount of generated angiotensin I for 1 hr at $37\,℃$ in the presence of excess rat angiotensinogen. The procedure was performed according to instructions for Angiotensin ^{125}I KIT (Immunotech, Czech Republic). Angiotensinogen substrate was prepared from bilaterally nephrectomized male rats. Twenty four hours after the surgery, animals were sacrificed by decapitation and the blood was collected into vials containing 0.5 mol/L ethylenediaminetetraacetic acid (EDTA), pH 8.0 in the amount of approximately 1/10th of the total blood volume. The blood was centrifuged 20 min at $2000 \times g$ and the plasma was used as the source of angiotensinogen. Angiotensin converting enzyme activity was assayed separately in homogenates of adipocytes and stromal-vascular tissue with hippuryl-L-histidyl-L-leucine[glycine-1-^{14}C]-(HHL) as the substrate for the enzyme (Rorhbach 1978) as previously reported (Heemskerk et al. 1999).

In our experiments, the gene expression of angiotensinogen, renin, ACE and AT_1 receptor was detected in the stromal-vascular fraction of adipose tissue. In adipocytes, the same components of the renin-angiotensin system were detected, except of ACE (Figure 1). Angiotensinogen mRNA in adipose tissue has already been detected by several authors (Frederich et al. 1992; Harp and DiGirolamo 1995; Engeli et al. 1999). Although the liver is traditionally considered to be the main source of angiotensinogen, the present study and also results of other investigators (Frederich et al. 1992; Harp and DiGirolamo 1995) suggest that adipose tissue can also considerably contribute to the circulating levels of AGT. In addition, the adipocyte AGT expression is nutritionally regulated. Fasting decreased and refeed-
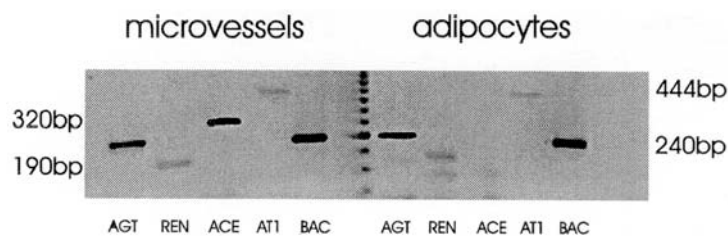

Figure 1. PCR fragments of angiotensinogen (AGT; 263 bp), renin (REN; 190 bp), angiotensin-converting enzyme (ACE; 320 bp) AT_1 receptor (AT_1; 444 bp) and beta-actin (BAC; 240 bp) genes in stromal-vascular fraction (microvessels) and adipocytes of rat fat tissue. The 50 base-pair ladder is in the central line.

ing increased the mRNA and protein levels of AGT. In the ob/ob mouse, which serves as a model of obesity, the AGT secretion was also increased in comparison to control non-obese animals (Frederich et al. 1992). Other studies also indicated higher levels of plasma AGT in obese patients as well as in obese rats (Tamura et al. 1997; Umemura et al. 1997). The presence of higher levels of AGT in blood or adipose tissue of obese humans and animals suggests the possible role of RAS in adipogenesis. Renin activity or renin-like activity was observed in adipose tissue by several authors (Harp and DiGirolamo 1995; Engeli et al. 1999), but none of them have succeeded in detecting renin mRNA. In our experiments, renin mRNA was detected in both, adipocytes and the stromal-vascular fraction of the adipose tissue (Figure 1). On the other hand, renin activity was found mainly in adipocytes (Table 2). The almost undetectable renin activity in the stromal-vascular fraction is possibly accounted for by less conversion of prorenin to renin.

Table 2. Renin activity and ACE activity in adipose tissue fractions (mean $\pm$ S.E.M.; * Significantly different from stromal-vascular fraction; $p < 0.05$).

Tissue fraction	Renin activity (ng Ang I/ml/hr/100μg protein)		ACE activity (pmol/hr/100μg protein)	
Adipocyte	11.3 ± 3.3	$(n = 3)$*	13.5 ± 5.6	$(n = 3)$*
Stromal-vascular	0.7 ± 0.2	$(n = 3)$	443 ± 125.2	$(n = 4)$

Similarly to human adipose tissue (Engeli et al. 1999), ACE mRNA was exclusively expressed in the stromal-vascular fraction of rat adipose tissue. In agreement with Crandall et al. (1992) and Harp and DiGirolamo (1995), ACE activity was mainly observed in the stromal-vascular fraction. Some minor activity was also detected in adipocytes (Table 2) which, however, could also be of vascular origin.

The observation of AT_1 receptor gene expression in adipose tissue is in line with our results based on immunoblot (Zorad et al. 1995) and with the binding studies of Crandall et al. (1993,1994). To the best of our knowledge, the present work provides the first evidence for the presence of AT_1 receptor mRNA in the stromal-vascular fraction of fat tissue. Additional experiments are needed to reveal the function of AT_1 receptors in fat tissue vessels.

Considering conditions when adipose tissue expresses its own RAS, adipose tissue may be fully independent of circulating RAS and may be able to generate angiotensin II for its own autocrine or paracrine regulations. Recent studies indicate that the proliferative effect of Ang II is mediated by AT_1 receptors. Ang II stimulated hyperplasia in smooth muscle cell culture and cardiomyocytes (Geisterfer et al. 1990; Paquet et al. 1990) and PC12 cells which expressed recombinant AT_1 receptor (Ozawa et al. 1998). Possibly, under the condition of higher expression

of angiotensinogen in adipocytes and its subsequent conversion to Ang II, higher levels of Ang II may have a proliferative effect on adipocytes through AT_1 receptors and thus contribute to adipogenesis.

In summary, we could demonstrate that all components of the renin-angiotensin system (AGT, renin, ACE, AT_1 receptors) are expressed in rat epididymal fat tissue. We further localised the AGT, renin and AT_1 expression in both, adipocytes and the stromal-vascular fraction, while ACE mRNA was present exclusively in the stromal-vascular fraction. The physiological relevance of the renin-angiotensin system in the adipose tissue is currently not clear; it may be suggested to play a role in metabolic pathways or in the regulation of blood flow through the tissue. It may also be implicated in adipogenesis and thus participate in the development of obesity and the metabolic syndrome.

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