Minireview

The Role of Angiotensin II and its Receptors in Regulation of Adipose Tissue Metabolism and Cellularity

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Abstract. Angiotensin II exerts its action via at least two distinct receptor subtypes designated AT_1 and AT_2. AT_1 receptors seem to be responsible for most of the known angiotensin II effects while the role of AT_2 receptors is not yet clear. Adipocytes of adult rats express exclusively the AT_1 subtype. Angiotensin II stimulates prostacyclin release in adult rat adipocytes and in mouse preadipocytes. In the latter prostacycline release is completely blocked by an AT_2 receptor antagonist. Adipocyte angiotensin II receptors seem to be regulated by age and fat mass. Blockade of these receptors by an AT_1 antagonist seems to prevent adipose tissue hypertrophy. Moreover, adipose tissue contains all the main components of the renin-angiotensin system such as angiotensinogen, angiotensin converting enzyme, angiotensin II and angiotensin II receptors. Angiotensinogen expression in adipocytes is stimulated by a high fat diet concurrent with enlargement of fat mass, associated with insulin resistance. Angiotensin converting enzyme inhibitors improve insulin sensitivity. Taken together, there is evidence of interaction between insulin and angiotensin II in regulation of adipose tissue metabolism and cellularity. Clarification of these interactions could lead to significant progress in pharmacological treatment of obesity and its comorbidity.

Key words: Angiotensin receptor subtypes — Renin-angiotensin system — Adipocytes — Obesity — Insulin resistance

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Introduction

Numerous dietary, genetic and hormonal factors are known to regulate the amount of adipose tissue in mammals (Hollenberg 1990; Kissebah and Krakower 1994). Excess of adipose tissue mass, obesity, is often associated with noninsulin dependent diabetes mellitus and hypertension (Hollenberg 1990). Recent evidence has implicated angiotensin II (A II) in the regulation of adipose tissue cellularity (Crandall et al.1994b; Darimont et al. 1994). This idea is supported by the presence of the main components of the renin – angiotensin system (RAS) in adipose tissue. In the rat, adipose tissue seems to be the largest source of angiotensinogen (AOGEN), a precursor of angiotensin I (A I) (Frederich et al. 1992). The A I is converted to A II by angiotensin converting enzyme (ACE), a dipeptidyl carboxypeptidase, which is expressed in the stroma – vascular tissue of fat pads as well (Crandall et al. 1992). Moreover, A II binding sites have recently been characterized in membranes of adipocytes (Crandall et al. 1993).

Generally, A II binds to two structurally and pharmacologically different receptor subtypes AT_1 and AT_2 (Bottari et al. 1993). So far the AT_1 subtype has been detected in rat adipocytes (Crandall et al. 1993), while AT_2 was found in mouse preadipocytes (Darimont et al. 1994). A II, a well-known vasoconstrictor, seems in addition to regulate growth and development (Timmermans et al. 1993). Adipose tissue is one of the few tissues capable of expanding through most of adult life (Klyde and Hirsh 1979). The presence of many components of the RAS in adipose tissue and the possible function of A II in growth and development led to investigate the role of components of the RAS, especially A II and A II receptors, in the regulation of adipocyte metabolism and adipose tissue cellularity.

Mechanism of A II action

A II, an octapeptide, exerts its biological action through two main subtypes of receptors. These receptor subtypes are structurally and biochemically well characterized and they both have been cloned (Murphy et al. 1991; Sasaki et al. 1991; Kambayashi et al. 1993; Mukoyama et al. 1993). The A II receptor subtype, AT_1, is a member of the G-protein-coupled seven-transmembrane receptor class containing 359 amino acids (Murphy et al. 1991; Sasaki et al.1991; Griendling et al. 1993). In the rat, but not in humans two different AT_1 receptor isoforms termed AT_{1A} and AT_{1B} were found (Chiu et al. 1994). The amino acid sequences of these two receptor isoforms have 96% identity. The only functional consequence of the small difference in amino acid sequence is differential regulation of isoreceptor gene expression (Chiu et al. 1994). The AT_1 receptor subtype seems to mediate most of the known A II actions such as smooth muscle contraction, aldosterone synthesis and secretion, neuropeptide secretion, increase of thirst and salt appetite and

renal sodium reabsorption (Bottari et al. 1993). This A II receptor subtype is believed to be coupled to at least two different G proteins, Gi and Gq (Bottari et al. 1993). Activation of Gi by A II inhibits adenylate cyclase in some tissues (Pobiner et al.1985). Activation of Gq or more specifically, activation of some so far unknown member of the Gq family by A II leads to stimulation of phospholipase C (phosphoinositidase C) which cleaves phosphatidylinositol 4,5 bisphosphate (PIP_2), originated in phosphatidylinositol, into inositol-1,4,5,-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG) (Beridge and Irvine 1989). IP_3 causes an increase of intracellular calcium concentration whereas DAG activates protein kinase C (PKC). In addition, AT_1 mediated stimulation of adenylate cyclase (Missale et al. 1989), phospholipase A_2 (Pfeilschifter 1989), and phospholipase D has been described in some tissues (Pfeilschifter et al.1992).

The other A II receptor subtype, AT_2, seems to belong to the unique family of seven-transmembrane receptors not coupled to G-proteins (Mukoyama et al.1993). It consists of 363 aminoacids with 32% homology to the AT_1 receptor. The function of AT_2 is not yet known. AT_2 binding sites are more abundant in embryonic and neonatal than adult tissues (Bottari et al. 1993). This may suggest that A II via AT_2 receptors is involved in regulation of differentiation and growth. This conclusion is strengthened by recent findings of Stoll et al. (1995) that AT_2 receptors mediate inhibition of proliferation of coronary endothelial cells. Only sparse data exist concerning AT_2 signal transduction. These suggest AT_2 coupling to particulate guanylate cyclase or phosphotyrosine phosphatase (Bottari et al. 1992; Griendling et al. 1993; Kambayashi et al. 1993).

Besides different structures of AT_1 and AT_2 receptors the pharmacology of ligand binding is the most apparent difference between these receptors. Currently a number of highly selective AT_1 and AT_2 nonpeptide antagonists/ligands have been developed (Timmermans et al. 1993; Chiu et al. 1994). Losartan {2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[2'(1H-tetrazol-5-yl)biphenyl-4-yl-methyl]imidazole} and other losartan-like compounds are highly selective for the AT_1 receptor displaying about 10,000-fold greater affinity than for AT_2 (Chiu et al. 1994). Losartan is able to block virtually all of the physiological effects of A II. In addition, the agonist binding to AT_1 receptors is sensitive to dithiothreitol (DTT) (Whitebread et al.1989; Tsutsumi et al. 1992) and the nonhydrolysable analogs of GTP (Dudley et al. 1990). In turn, AT_2 receptor binding is stimulated by DTT, and generally the AT_2 receptors are insensitive to GTP analogs (Dudley et al. 1991; Zorad et al. 1994). A II binding to AT_2 receptors is insensitive to losartan, but is blocked by AT_2-selective ligands such as peptidic CGP 42112A [nicotinic acid-Tyr-(N^E-benzyloxycarbonyl-Arg)Lys-His-Pro-Ile-OH] or nonpeptidic PD 123177 {1-(4-amino-3-methylphenyl)methyl-5-diphenylacetyl-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid-2HCl} (Timmermans et al. 1993; Heemskerk et al. 1993; Zorad et al. 1994). The above agents are about 1000–3500-fold more selec-

tive for AT_2 than for AT_1 (Chiu et al. 1994). The use of AT_1 and AT_2 selective ligands is the simplest approach to discriminating between these receptor subtypes, especially since the AT_2 receptor response is not yet known.

Some A II receptors seem to have atypical binding properties with regard to interaction with subtype selective ligands, DTT and analogs of GTP (Timmermans et al. 1993). E.g., they are either not blocked by AT_1/AT_2 blockers or are blocked by AT_2 blockers and are sensitive to nonhydrolyzable analogs of GTP (Tsutsumi and Saavedra 1992). The biological function of "atypical" A II receptors is not know.

Renin-angiotensin system in adipose tissue

Adipose tissue is one of the "nonclassical" RAS tissues expressing high levels of AOGEN (Cassis et al 1988). AOGEN mRNA and secretion in white adipocytes is nutritionally regulated unlike in the liver (Frederich et al. 1992). During fasting the AOGEN mRNA and release from adipocytes is decreased. Refeeding and obesity increase AOGEN secretion from adipocytes (Frederich et al. 1992).

ACE is another RAS component present in adipose tissue, specifically in the stroma-vascular fraction (Crandall et al.1992). In addition A II receptors have recently been identified in rat epididymal adipocytes (Crandall et al. 1993) and mesenteric and retroperitoneal fat depots (Crandall et al. 1994a). Adipocytes from aged rats seem to exclusively express the AT_1 subtype of A II receptors (Fig. 1). However, Darimont et al. (1994) showed AT_2 receptors to be present in differentiated adipocytes derived from an Ob1771 mouse preadipocyte clonal line upon stimulation by A II. The stimulated adipocytes release a prostacyclin (PGI_2) which plays a key role in preadipose cell differentiation *in vitro* (Vassaux et al. 1992). Thus the nutritionally induced increase of local production of AOGEN by mature adipocytes may, via increased local levels of A II and PGI_2, lead to hyperplasia of adipose tissue, once the adipocytes reach a critical size (Kral 1976; Faust et al.1978). The A II stimulated prostacyclin release in cultured mouse adipocytes was totally blocked by the specific AT_2 ligand PD 123 177 (Darimont et al. 1994). On the other hand Crandall et al. (1994b) observed A II stimulated release of $PGF_{1\alpha}$, a stable metabolite of prostacyclin, in adult rat adipocytes expressing exclusively AT_1 receptors. These conflicting results suggest either species differences or age differences. The latter is supported by the higher abundance of AT_2 subtype in fetal and young tissues (Saavedra 1992; Bottari et al. 1993).

The results of Darimont et al. (1994) suggest a role of A II in adipose tissue hyperplasia, while the results of Crandall et al. (1994b) show the possible role of this hormone in hypertrophy of fat tissue. Losartan, an AT_1 specific antagonist, inhibited the age dependent increase in adipocyte size (Crandall et al. 1994b). In the same experiment the number of AII receptors in adipocyte plasma membranes

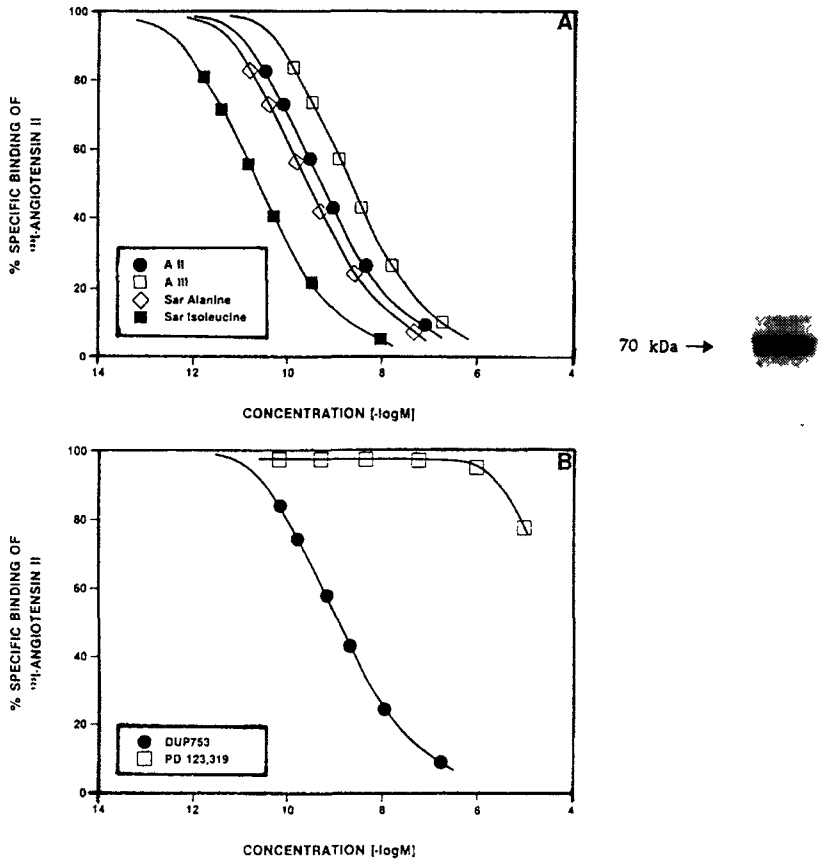

Figure 1. Left side: Displacement of ^{125}I-A II bound to fat cell membranes by A II, agonists and antagonists. (*A*) Compounds that are nonselective for receptor subtypes; (*B*) Selective antagonists for AT_1 (DuP 753 = losartan) and AT_2 (PD 123319), respectively [from Crandall et al. (1993); with permission]. Right side: Western-Blot analysis of membranes from rat epididymal fat. The AT_1 specific antibody was prepared as described in Zelezna et al. (1992).

was significantly lower in adult, obese than in young, lean rats. Hypothetically the increased adipose tissue mass in adult, obese rats produces more A II locally which may downregulate the A II receptors in adipocytes.

Taken together the recent experimental data underline the possible importance of AII and AII receptors in the regulation of adipocyte metabolism and in adipose tissue growth and development.

Therapeutic implications related to adipose tissue RAS

Since excessive enlargement of adipose tissue is an important risk factor for the development of prevalent disorders such as insulin resistance, diabetes and hypertension (Kissebah and Krakower 1994), elucidation of factors regulating adipose tissue growth is of major interest. Recent experimental data suggest a role for A II in this process (Crandall et al. 1994b; Darimont et al. 1994). Confirmation of the regulatory role of A II in development of adipose tissue and understanding of the function of RAS in this tissue may provide an important tool for treating obesity. Indeed, it was already shown that the losartan treatment of rats results in significant decrease of adipocytes cell volume (Crandall et al. 1994b). Moreover, clinical studies suggest that ACE inhibitors used as antihypertensive agents increase insulin sensitivity in patients with diabetes and hypertension (Connell and McLellan 1991; Lewis et al. 1993; Torlone et al. 1993; Okša et al. 1994). Interestingly, in healthy individuals A II infusion also enhanced insulin sensitivity (Fliser et al. 1993; Raymond and DiPette 1993). These data and the fact that fat tissue is one of the main targets for insulin suggest an interaction between the RAS and insulin receptors and the glucose transporter system in fat tissue. In addition, with increased use of losartan clinically, data on the effect of AT_1 receptor blockade on glucose metabolism, seem especially important in hypertensive diabetics.

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