Brazilian Journal of Medical and Biological Research (1998) 31: 1349-1352
ISSN 0100-879X Short Communication

The hyperglycemia induced by angiotensin II in rats is mediated by AT_1 receptors

L.J.C. Machado[1], U. Marubayashi[2], A.M. Reis[2] and C.C. Coimbra[2]

[1]Departamento de Clínica Médica, Faculdade de Medicina, and [2]Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

Abstract

We have shown that the renin-angiotensin system (RAS) is involved in glucose homeostasis during acute hemorrhage. Since almost all of the physiological actions described for angiotensin II were mediated by AT_1 receptors, the present experiments were designed to determine the participation of AT_1 receptors in the hyperglycemic action of angiotensin II in freely moving rats. The animals were divided into two experimental groups: 1) animals submitted to intravenous administration of angiotensin II (0.96 nmol/100 g body weight) which caused a rapid increase in plasma glucose reaching the highest values at 5 min after the injection (33% of the initial values, $P<0.01$), and 2) animals submitted to intravenous administration of DuP-753 (losartan), a non-peptide antagonist of angiotensin II with AT_1-receptor type specificity (1.63 µmol/100 g body weight as a bolus, *iv*, plus a 30-min infusion of 0.018 µmol 100 g body weight^{-1} min^{-1} before the injection of angiotensin II), which completely blocked the hyperglycemic response to angiotensin II ($P<0.01$). This inhibitory effect on glycemia was already demonstrable 5 min (8.9 ± 0.28 mM, angiotensin II, N = 9 *vs* 6.4 ± 0.22 mM, losartan plus angiotensin II, N = 11) after angiotensin II injection and persisted throughout the 30-min experiment. Controls were treated with the same volume of saline solution (0.15 M NaCl). These data demonstrate that the angiotensin II receptors involved in the direct and indirect hyperglycemic actions of angiotensin II are mainly of the AT_1-type.

Correspondence
C.C. Coimbra
Departamento de Fisiologia e Biofísica, ICB-UFMG
Av. Antônio Carlos, 6627
31270-901 Belo Horizonte, MG
Brasil
Fax: +55-31-499-2924
E-mail: coimbrac@mono.icb.ufmg.br

Research supported by PRPq-UFMG, CAPES, CNPq, FINEP, FAPEMIG and FUNDEP.

Received December 16, 1997
Accepted July 24, 1998

Key words
- Glycemia
- Angiotensin II
- DuP-753
- AT_1 receptor

In addition to affecting fluid volume, electrolytes and hemodynamic states, the renin-angiotensin system (RAS) is also involved in the regulation of metabolic and endocrine function, especially blood glucose homeostasis (1-3). Several *in vitro* hepatocyte studies have shown that angiotensin II stimulates glycogen phosphorylase activity (4-6) and gluconeogenesis (7-10). Recently, we have shown that RAS involvement in blood glucose regulation is of physiological significance, with angiotensin II producing a dose-dependent hyperglycemic response (1). In contrast, intravenous infusion of an angiotensin II peptide-analog antagonist, [1-sar,8-thr]-angiotensin II (sarthran), had an

inhibitory effect on hemorrhage hyperglycemia (1,2). These were the first data demonstrating that the RAS elicits a physiological glycemic response to angiotensin, in addition to activating the sympathetic nervous system and adrenomedullary secretion (2,3). Therefore, the next step was to determine which type of angiotensin II receptor is involved in this well-confirmed hyperglycemic action of angiotensin II. Since most of the physiological functions described for angiotensin II are mediated by the AT_1-receptor type (11), the present study was designed to investigate the effect of DuP-753 (losartan), a non-peptide AT_1-selective antagonist (11,12), on the hyperglycemic response to intravenous injection of angiotensin II.

Adult male Wistar rats (12-14 weeks) had free access to Purina rat chow and tap water and were housed under controlled temperature with 14 h of light (5:00-19:00 h) per day. At the age of 11 weeks, the rats were placed in individual cages and handled frequently. One week later, they were anesthetized with ether and a silastic catheter was inserted through the jugular vein into the right atrium for blood sampling. This catheter was filled with saline solution and rinsed every other day with heparinized saline solution (25 IU/ml). All animals were allowed to recover for one week before being utilized in the experiments.

On the day of the experiment, the rats had their venous catheter connected to a peristaltic pump 1 h prior to intravenous infusion of angiotensin II (Sigma, St. Louis, MO). After 30 min, DuP-753 (Du Pont Merck Pharmaceutical Company, Wilmington, DE) was administered intravenously over a 30-min period (1.63 µmol/100 g body weight as a bolus plus a continuous infusion of 0.018 µmol 100 g body weight^{-1} min^{-1}). Controls submitted to 30-min saline infusion before angiotensin II injection (Sal/Ang II group) were treated with the same volume (0.2 ml as a bolus plus an infusion of 0.007 ml 100 g body weight^{-1} min^{-1}) of saline solution (0.15 M NaCl). At time zero, losartan or saline infusion was stopped and angiotensin II (0.96 nmol/100 g body weight) or saline (0.15 M NaCl, 0.2 ml/100 g body weight) was injected over a period of 2 min. Blood samples (0.4 ml) were col-

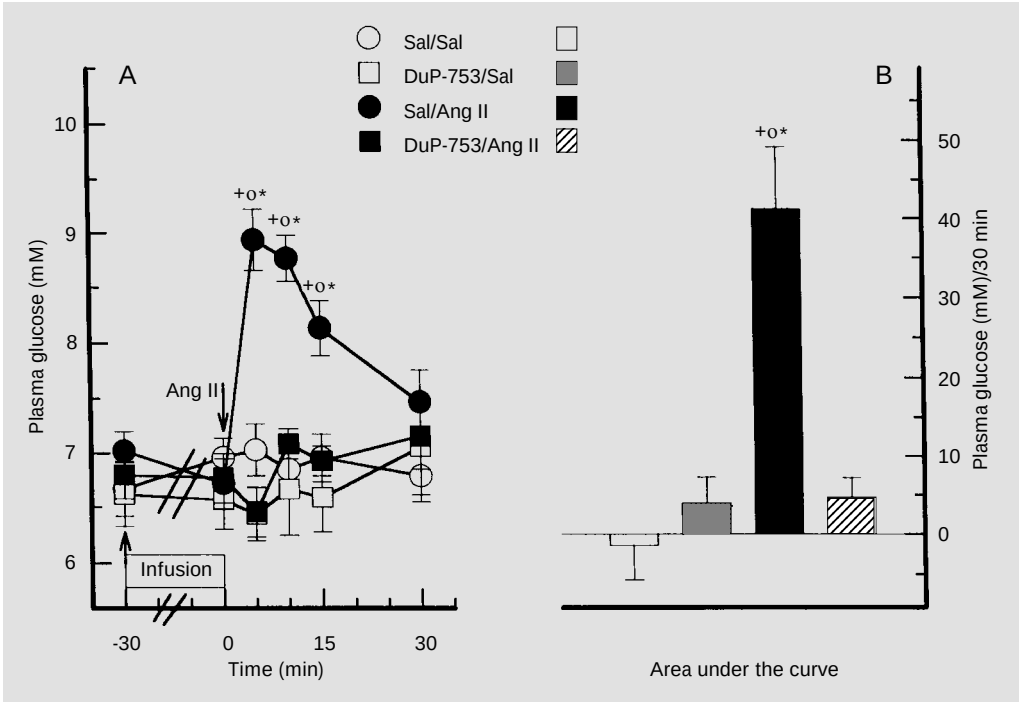

Figure 1 - *A*, Effect of pretreatment with losartan (DuP-753, 1.63 µmol/100 g body weight as a bolus, *iv*, plus continuous infusion of 0.018 µmol 100 g body weight^{-1} min^{-1} for 30 min) or saline (Sal, 0.15 M NaCl) on the hyperglycemia induced by angiotensin II injection (Ang II, 0.96 nmol/100 g body weight). *B*, Integrated areas under the glucose curves (shown in panel A). Each point represents the mean ± SEM of N = 7-11 observations. $^{+}$P<0.01 *vs* the Sal/Sal group; ^{o}P<0.01 *vs* the DuP-753/Sal group; *P<0.01 *vs* the DuP-753/Ang II group (Newman-Keuls test).

lected at -30 min (immediately before losartan or saline pretreatment) and 0, 5, 10, 15 and 30 min after the injection of angiotensin II or saline. The volume was replaced with saline solution after each sample. The experiments were done between 12:00 and 17:00 h. Blood was centrifuged at 4°C and plasma was stored at -20°C until the time for the glucose assay, carried out in duplicate by the oxidase method (GodAna, Labtest, BR, Lagoa Santa, MG). The data are reported as means ± SEM. The integrated area under the glucose curve was calculated by the trapezoidal rule. Differences between groups were determined by analysis of variance followed by the Newman-Keuls test. Glycemia after angiotensin II injection was compared to basal values by the paired Student *t*-test. A probability of $P<0.05$ was considered to be significant.

As illustrated in Figure 1A, following the injection of 0.96 nmol/100 g body weight of angiotensin II (Sal/Ang II group, 9 rats) there was an immediate increase in plasma glucose levels, reaching the highest value at 5 min after injection (8.9 ± 0.28 mM, at 5 min *vs* 6.7 ± 0.23 mM, basal value), when the increase was about 33% of the initial values ($P<0.01$). At 10 min the values were still high (16.6%, $P<0.01$), and at 30 min post-injection plasma glucose levels were returning to normal. The increase of plasma glucose following angiotensin II injection was completely blocked ($P<0.01$) by infusion of the angiotensin II antagonist losartan (DuP-753/Ang II group, 11 rats). This effect of losartan infusion persisted throughout the 30-min experimental period. Plasma glucose of saline-pretreated (Sal/Sal group) and losartan-pretreated (DuP-753/Sal group) rats did not change during control tests without angiotensin II injection (Figure 1A,B).

The present data show that the hyperglycemia induced by angiotensin II is completely blocked by DuP-753, a non-peptide antagonist of angiotensin II with AT_1-receptor type specificity (11,12). In fact, this receptor type seems to mediate the actions of angiotensin II in the liver that contains only angiotensin II receptors which can be blocked by DuP-753 (11,13,14). It has been shown recently that angiotensin II increases hepatic glucose production by a receptor-mediated mechanism that is not related to the pressor response to the hormone (15). Angiotensin II induces transduction signs (phosphoinositide turnover and calcium mobilization) and activates glycogen phosphorylase and adenylate cyclase through AT_1 receptors in hepatocytes (13,14). In addition, losartan has been shown to block the increased production of glucose by angiotensin II infused during a single-pass perfusion of rat liver (16). However, we have recently demonstrated that the hyperglycemic response to angiotensin II is also dependent on sympathetic adrenomedullary system activation. Therefore, a hyperglycemic response to angiotensin II attributed to this indirect action of the peptide could occur despite the blockade of the hepatocyte AT_1 receptors by losartan administration. It is important to stress that the stimulatory actions of angiotensin II on adrenal catecholamine release are also inhibited by losartan, despite the predominance of the AT_2 receptor type in the adrenal medulla (12,17,18). Therefore, the results of these studies are in agreement with our present data and previous studies (1-3), and indicate that the hyperglycemic effect of angiotensin II produced by its stimulatory actions on the sympathetic adrenomedullary system and on hepatic glucose output is losartan sensitive.

In summary, the present results show that the angiotensin II receptors involved in the direct and indirect hyperglycemic actions of the hormone are both mainly of the AT_1-type.

References

1. Machado LJC, Mihessen-Neto I, Marubayashi U, Reis AM & Coimbra CC (1995). Hyperglycemic action of angiotensin II in freely moving rats. *Peptides*, 16: 479-483.
2. Machado LJC, Marubayashi U, Reis AM & Coimbra CC (1995). Effect of [1-Sar,8-Thr]-angiotensin II on the hyperglycemic response to hemorrhage in adrenodemedullated and guanethidine-treated rats. *Regulatory Peptides*, 60: 69-77.
3. Mihessen-Neto I, Reis AM, Marubayashi U & Coimbra CC (1996). Effect of sympathoadrenal blockade on the hyperglycemic action of angiotensin II. *Neuropeptides*, 30: 303-308.
4. Campanile CP, Crane JK, Peach MJ & Garrison JC (1982). The hepatic angiotensin II receptor. I. Characterization of the membrane-binding site and correlation with physiological response in hepatocytes. *Journal of Biological Chemistry*, 257: 4951-4958.
5. Hems DA (1977). Short-term hormonal control of hepatic carbohydrate and lipid catabolism. *FEBS Letters*, 80: 237-245.
6. Keppens S & De Wulf H (1976). The activation of liver glycogen phosphorylase by angiotensin II. *FEBS Letters*, 68: 279-282.
7. Hothi SK, Leach RP & Titheradge MA (1988). Comparison of the effects of [leucine]enkephalin and angiotensin on hepatic carbohydrate and cyclic nucleotide metabolism. *Biochemical Journal,* 249: 669-676.
8. Kneer NM & Lardy HA (1983). Regulation of gluconeogenesis by norepinephrine, vasopressin, and angiotensin II: A comparative study in the absence and presence of extracellular Ca^{2+}. *Archives of Biochemistry and Biophysics*, 225: 187-195.
9. Sistare FD & Haynes RC (1985). Estimation of the relative contributions of enhanced production of oxaloacetate and inhibition of pyruvate kinase to acute hormonal stimulation of gluconeogenesis in rat hepatocytes. *Journal of Biological Chemistry*, 260: 12761-12768.
10. Whitton PD, Rodrigues LM & Hems DA (1978). Stimulation by vasopressin, angiotensin and oxytocin of gluconeogenesis in hepatocyte suspensions. *Biochemical Journal,* 176: 893-898.
11. Timmermans PBMWM, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler RR, Saye JAM & Smith RD (1993). Angiotensin II receptor antagonists. *Pharmacological Reviews*, 45: 205-251.
12. Wong PC, Hart SD, Zaspel AM, Chiu AT, Ardecky RJ, Smith RD & Timmermans PBMWM (1990). Functional studies of nonpeptide angiotensin II receptor subtype-specific ligands: Dup 753 (AT_1) and PD123177 (AT_2). *Journal of Pharmacology and Experimental Therapeutics*, 255: 584-592.
13. Bauer PH, Chiu AT & Garrison JC (1991). DuP 753 can antagonize the effects of angiotensin II in rat liver. *Molecular Pharmacology*, 39: 579-585.
14. García-Sáinz JA, Gonzáles-Espinosa C & Olivares-Reyes JA (1995). Differences between rapid and long-term actions of angiotensin II in isolated rat hepatocytes. Effects on phosphorylase A activity and c-fos expression. *Archives of Medical Research*, 26: S189-S193.
15. Rao RH (1996). Pressor doses of angiotensin II increase hepatic output and decrease insulin sensitivity in rats. *Journal of Endocrinology*, 148: 311-318.
16. Reisenleiter F, Katz N & Gardemann A (1996). Control of hepatic carbohydrate metabolism and haemodynamics in perfused rat liver by arterial and portal angiotensin II. *European Journal of Gastroenterology and Hepatology*, 8: 279-286.
17. Song K, Zhuo J, Allen AM, Paxinos G & Mendelsohn FAO (1991). Angiotensin II receptor subtypes in rat brain and peripheral tissues. *Cardiology*, 79: 45-54.
18. Chiu AT, Herblin WF, McCall DE, Ardecky RJ, Carini DJ, Duncia JV, Pease LJ, Wong PC, Wexler RR, Johnson AL & Timmermans PBMWM (1989). Identification of angiotensin II receptor subtypes. *Biochemical and Biophysical Research Communications*, 165: 196-203.