The hyperglycemia induced by angiotensin II in rats is mediated by AT₁ receptors

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 AT₁ receptors, the present experiments were designed to determine the participation of AT₁ 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 AT₁-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 AT₁-type.

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 (sartrhan), 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 AT1-receptor type (11), the present study was designed to investigate the effect of DuP-753 (losartan), a non-peptide AT1-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 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·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·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-
lected at -30 min (immediately before losar-
tan 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 glu-
cose 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 return-
ing to normal. The increase of plasma glu-
cose 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 lo-
sartan-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 hypergly-
cemia induced by angiotensin II is com-
pletely blocked by DuP-753, a non-peptide
antagonist of angiotensin II with AT_1-recep-
tor type specificity (11,12). In fact, this re-
ceptor 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
and glucose production by a receptor-mediated
mechanism that is not related to the pressor
response to the hormone (15). Angiotensin
II induces transduction signs (phosphoinosi-
tide turnover and calcium mobilization) and
activates glycogen phosphorylase and aden-
ylate cyclase through AT_1 receptors in hepa-
tocytes (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). How-
ever, we have recently demonstrated that the
hyperglycemic response to angiotensin II is
also dependent on sympathetic adrenomed-
ular system activation. Therefore, a hyper-
glycemic response to angiotensin II attrib-
uted to this indirect action of the peptide
could occur despite the blockade of the hepa-
tocyte AT_1 receptors by losartan administra-
tion. It is important to stress that the stimula-
tory 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


