Effect of increasing doses of angiotensin II infused into normal and hypertensive Wistar rats on low density lipoprotein and fibrinogen uptake by aortic walls

(blood pressure)

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ABSTRACT The effect of 6 days’ s.c. infusions of angiotensin II at increasing doses was determined on the uptake of rat or human low density lipoprotein (LDL) and of human fibrinogen by aorta in normal and spontaneously hypertensive rats. Rat or human LDL or human fibrinogen was injected i.v. 5 days after the start of infusion, and 24 hr later the radioactivity of aortic walls was determined. Body weight was almost constant in control rats and moderately decreased in a dose-dependent way by angiotensin II. Diastolic blood pressure decreased slightly over 6 days in control rats and increased transiently at the lowest dose of angiotensin II and progressively with two higher concentrations. All three angiotensin II concentrations significantly increased the uptake of rat and human LDL and of fibrinogen by aorta. The increase was dose related for rat LDL but not for human LDL or fibrinogen. In spontaneously hypertensive rats of the same age in which blood pressure was higher than in angiotensin II-infused rats, protein uptakes were not increased. The blood content of aortic walls was negligible and not altered by angiotensin II. Therefore, the uptake of atherogenic plasma proteins by rat aorta is increased by angiotensin II, but this effect may be independent of its pressor action.

In a preceding paper (1) we provided evidence that angiotensin II, like noradrenaline (2) and adrenaline (3, 4), increases the uptake of low density lipoprotein (LDL) and fibrinogen by the walls of large arteries in rabbits and rats. This effect was demonstrable at catecholamine or angiotensin II concentrations that caused moderate rises in blood pressure and in time spans of a few hours (5).

In this paper we provide evidence that infusion of angiotensin II in Wistar rats increases LDL and fibrinogen uptake even when the diastolic pressure is hardly affected—yet the uptake increases with higher blood pressures—and that the still higher blood pressure of spontaneously hypertensive rats (SHRs) is not associated with uptake greater than in controls.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing 300–350 g were fed ad libitum on standard diet. The rats were kept in wire-bottomed cages in an air-conditioned animal house with a light period lasting from 8 a.m. to 8 p.m.

Fibrinogen. Lyophilized human fibrinogen (KaviVitrum, Stockholm) was dissolved in physiological saline to give a concentration of 100 mg/ml.

Human LDL Preparation. Human blood was obtained by venepuncture from healthy students or laboratory personnel with lipid levels in the normal range. After mixing with 0.15 M EDTA, the blood was centrifuged at 800 × g for 30 min at 4°C. LDL (ρ = 1.019–1.063 g/ml) was separated by flotation after sequential centrifugation at 105,000 × g for 24 hr at 4°C (MSE Superspeed 65 in a 10 × 10 titanium angle rotor) on KBr solutions adjusted to final densities of 1.019 and 1.063 (6–8). The final LDL fraction was dialyzed exhaustively at 4°C against phosphate-buffered saline containing 0.3 mM EDTA at pH 7.5 to remove excess salts. Protein content was measured by bicinchoninic acid assay reagent (Pierce).

Rat LDL Preparation. The animals were killed by intraaortic puncture under pentobarbital anesthesia. The blood of each rat was collected on 4% Na2EDTA and centrifuged at 2200 × g for 20 min at 4°C. Pooled plasma from four rats was used for lipoprotein isolation as described (9).

Preparation and Labeling of LDL and Fibrinogen. An adduct of cellobiose and tyramine (TC) was radioiodinated with 125I or 131I and attached covalently to LDL or fibrinogen, respectively. The labeled TC remains in situ when the lipoprotein or fibrinogen undergo degradation, so that the radioactivity is a measure of the proteins that were present in the tissues (10). The labeled proteins were dialyzed exhaustively in cold phosphate-buffered saline containing 2 mM EDTA at pH 7.5 and sterilized by filtration. The TC-labeled LDL (125I-TC-LDL) or fibrinogen (131I-TC-fibrinogen) had a specific activity of 100–400 cpm/ng of protein and free 125I content of 1–4% for the LDL fraction and of 50–150 cpm/ng of protein and free 131I content of 1–3.5% for the fibrinogen preparation.

Surgical Procedure. Rats were anesthetized with i.m. Hypnorm (Janssen), 0.3 ml/kg, and i.p. methyl dazepin-one (Diazepam, Phoenix Pharmaceuticals, St. Joseph, MO), 2.5 mg/kg. The surgical procedure was as follows: A ventral incision was made in the neck to expose the common carotid. A 0.4-mm (i.d.), 0.8-mm (o.d.) polyethylene catheter (Portex, Portex, France) was placed in the carotid artery, which was accessed via a small incision on the dorsal aspect of the neck. The catheter was flushed daily with 0.3 ml of hirudin solution, 10 µg/ml. The following day, a small incision was made in the back of anesthetized rats (i.m. Hypnorm, 0.15 ml/kg) and osmotic minipumps (model 1007D; Alza) were implanted s.c. The pumps contained angiotensin II at concentrations of 4.9 mg/ml (ca. 5 mM), 9.8 mg/ml (ca. 10 mM), and 18.6 mg/ml (ca. 20 mM) or saline in control rats. The pumps delivered their contents at a rate of 0.5 µl/hr over 6 days. Five days after implanting the minipumps the rats were anesthetized with Hypnorm only, and labeled proteins (200–280 µg of each labeled protein per rat) were injected through the femoral vein. Twenty-four hours later the rats were killed by an overdose of pentobarbital. The whole length of the aorta was exposed from

Abbreviations: LDL, low density lipoprotein; SHR, spontaneously hypertensive rat; TC, tyramine cellobiose; RBC, erythrocyte.

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the heart to the bifurcation and excised intact. Surrounding fat and
loose connective tissue were removed under a stereomicroscope
and the vessel was carefully cleaned and opened longitudinally.
The vessel was washed five times for 15 min each in 50 ml of ice-cold
saline followed by a wash of 5% KI in 0.9% saline and placed
in preweighed tubes. After counting radioactivity in a Packard
C5002 γ-counter, the tubes were dried to constant weight at 60°C
for at least 3 days. Results are expressed as ng of protein per
mg of dry weight. Body weight was determined 0, 2, 4, and 6 days
after minipump implantation. All procedures were approved by the
Animal Care and Safety Committee and conformed to guidelines estab-
lished by the British Home Office.

**Cr-Labeled Erythrocyte (RBC) Radioactivity in Tissues.**
Blood was mixed with acid/citrate/dextrose and some of the
supernatant citrate/plasma was removed before incubation with
**Cr. Sodium chromate (**Cr): 2.0 mCi; 1 Cl = 37 GBq;
(Amersham) was added slowly to the packed RBCs with continuous
gentle mixing. After 30 min of incubated at 37°C, ascobic acid
was added to reduce unbound chromate to the trivalent state.
After washing twice, resuspended labeled RBCs were injected i.v.
into the femoral vein and allowed to circulate for 1 hr (11, 12).
Blood samples were collected to determine the radioactivities of
the RBCs and the plasma. At the end of the experiment the whole
aorta was collected and washed to remove the blood, and the radioactivity
was determined.

**Blood Pressure Measurements.** The rats’ blood pressure
was determined from the carotid artery the day before and 2,
4, and 6 days after minipump implantation. The measurements
were made on the conscious, unrestrained animals by
connecting the indwelling catheter in the carotid artery to a
transducer coupled to a blood pressure Multitrace 2 recorder
5022 (Lectromed, Hertfordshire, UK), which measured heart
rate at the same time.

**Statistical Analysis.** The significance of differences among
groups of animals was assessed by the two-tailed Student’s t
for unpaired or paired samples. Plasma clearance rates of
each test group at each time point were tested by the
Mann-Whitney U test. A two-way ANOVA test was used to
compare means among different groups and to analyze intragroup
variation. In all statistical analyses, a P value of
<0.05 was considered significant. Results are shown as mean
± SEM.

**RESULTS**

**Body Weight.** In control rats and in SHRs the body weight
was almost constant over the 6-day experimental periods
(Table 1). Infusion of angiotensin II caused a progressive,
dose-dependent decrease in body weight to a maximum of
20% after 6 days on the highest dose (Table 1).

**Blood Pressure.** In control rats infused with saline the
diastolic blood pressure decreased progressively but not
significantly over 6 days (Table 2). Infusions of angiotensin II
at the lowest dose raised diastolic blood pressure transiently
only after 4 days. The two higher doses produced the
well-known progressive increase in blood pressure, reaching
about 173 mmHg after 6 and 4 days, respectively. In SHRs
the diastolic pressure was almost constant and higher at
191-202 mmHg than that produced by the largest dose of
angiotensin II (Table 2).

**Plasma Clearance.** The rates of clearance of the injected
proteins from the blood were compared in control rats with
rats infused with the middle dose (minipump concentration,
9.6 mM) of angiotensin II (Fig. 1). In control rats the plasma
clearance of rat LDL (t½ = 5 hr) was more rapid than that
of human LDL (t½ = 14 hr) (Fig. 1). The plasma clearances
of rat and human LDL were slightly but not significantly
accelerated by angiotensin II. Clearance measurements of
fibrinogen were more variable because of low specific activ-

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**Table 1. Effect of increasing concentrations of angiotensin II on body weight**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>274 ± 6</td>
<td>267 ± 4**</td>
<td>272 ± 5</td>
<td>270 ± 6</td>
<td>19</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>256 ± 2</td>
<td>240 ± 6**</td>
<td>240 ± 5**</td>
<td>239 ± 5**</td>
<td>12</td>
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<tr>
<td>mpc, 4.8 mM</td>
<td>269 ± 4</td>
<td>243 ± 7**</td>
<td>236 ± 5**</td>
<td>232 ± 5**</td>
<td>18</td>
</tr>
<tr>
<td>mpc, 9.6 mM</td>
<td>275 ± 6</td>
<td>252 ± 6**</td>
<td>252 ± 5**</td>
<td>220 ± 6**</td>
<td>22</td>
</tr>
<tr>
<td>mpc, 19.2 mM</td>
<td>252 ± 2</td>
<td>242 ± 4</td>
<td>245 ± 3</td>
<td>248 ± 3</td>
<td>15</td>
</tr>
</tbody>
</table>

The effect of saline (control, SHR) and increasing angiotensin II
concentrations infused s.c. by osmotic minipumps on the body
weight of conscious, unrestrained rats is shown. mpc, Minipump
concentration. *, P<0.05; **, P<0.01.

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**Table 2. Effect of increasing concentrations of angiotensin II on diastolic blood pressure**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>129 ± 4</td>
<td>126 ± 4</td>
<td>127 ± 4</td>
<td>123 ± 4</td>
<td>16</td>
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<tr>
<td>Angiotensin II</td>
<td>mpc, 4.8 mM</td>
<td>132 ± 3</td>
<td>139 ± 4</td>
<td>160 ± 8**</td>
<td>10</td>
</tr>
<tr>
<td>mpc, 9.6 mM</td>
<td>130 ± 4</td>
<td>147 ± 5**</td>
<td>145 ± 4**</td>
<td>173 ± 7*</td>
<td>16</td>
</tr>
<tr>
<td>mpc, 19.2 mM</td>
<td>143 ± 4</td>
<td>159 ± 4**</td>
<td>177 ± 8**</td>
<td>172 ± 6**</td>
<td>20</td>
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<tr>
<td>SHR</td>
<td>195 ± 7</td>
<td>191 ± 5</td>
<td>201 ± 5</td>
<td>202 ± 5</td>
<td>14</td>
</tr>
</tbody>
</table>

The effect of saline (control, SHR) and increasing angiotensin II
concentrations infused s.c. by osmotic minipumps on the diastolic
blood pressure of conscious, unrestrained rats is shown. mpc, Minipump
concentration. *, P<0.05; **, P<0.01.

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**Fig. 1. Plasma clearance of rat LDL (A), human LDL (B), human
fibrinogen (C), and human albumin (D) in Wistar rats during the final
24 hr of 6 days’ infusion with saline (C), n = 4) or angiotensin II (Φ, n = 4) at a minipump concentration of 9.6 mM.**
**DISCUSSION**

The results indicate that angiotensin II infused by implanted minipumps for 6 days into conscious unrestrained Wistar rats significantly increases the uptake by aortic walls of rat or human LDL or human fibrinogen injected i.v. 24 hr before the end of the angiotensin infusion. The validity of the conclusion that it is increased uptake rather than decreased elimination is based on the labeling technique in which the 125I or 131I tracer is attached to the proteins by TC, which is trapped intracellularly and persists in aortic walls as well as other tissues for >24 hr, during which the proteins themselves may be degraded and removed (10). Therefore, the radioactivities were a measure of the amount of the labeled proteins that had been taken up by the aortic walls. Infused angiotensin II had no significant effect on the rate of clearance of LDL and fibrinogen from the blood, so that the uptake effect of angiotensin II could not be accounted for by changes in the blood concentrations of the proteins. The possibility that the effect of angiotensin II might somehow have been due to its increasing the blood content of the aortic walls was excluded by experiments showing that the contribution to the tissues' radioactivities of the blood content was negligible and not significantly altered by angiotensin II infusions.

### Table 3. Fibrinogen uptake by aorta

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Angiotensin II mpc,* mM</th>
<th>SHR*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.8</td>
<td>9.6</td>
</tr>
<tr>
<td>1</td>
<td>100 ± 14</td>
<td>172 ± 10</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(7)</td>
</tr>
<tr>
<td>2</td>
<td>100 ± 14</td>
<td>271 ± 16</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(7)</td>
</tr>
<tr>
<td>3</td>
<td>100 ± 8</td>
<td>131 ± 11</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>4</td>
<td>100 ± 8</td>
<td>169 ± 5</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
</tr>
</tbody>
</table>

mpc, Minipump concentration. Shown is the aortic uptake of human 131I-Tc fibrinogen in the final 24 hr of 6 days' s.c. infusion of saline (control, n = 24; SHR, n = 15) and various angiotensin II concentrations (minipump concentrations: 4.8 mM, n = 12; 9.6 mM, n = 26; 19.2 mM, n = 22) by implanted minipumps in conscious normal Wistar rats and Wistar SHRs. n values are in parentheses. The significance of pooled experiments was analyzed by two-way ANOVA: dose F(3, 59) = 18.21; P < 0.01.

*Uptake as percentage of control.

**Fibrinogen Uptake.** The uptake of human fibrinogen was significantly increased by angiotensin II. In four experiments with fibrinogen the specific activities of the 131I-TC-labeled protein varied greatly, so that the results are expressed as percentage increases produced by angiotensin II compared with controls (Table 3).

**Uptake of LDL and Fibrinogen in SHRs.** In Wistar SHRs of the same age and slightly lower weight than the normal Wistar rats used in these experiments the diastolic blood pressure was 191–202 mmHg throughout the 6-day periods in which they were infused with saline. In these SHRs the uptakes of rat and human LDL and human fibrinogen were not significantly different from those of the controls (Figs. 2 and 3; Table 3).

**Blood Content of Rat Aortae.** In two experiments, RBCs taken from rats and labeled with 51Cr in vitro were reinjected i.v. From the 51Cr radioactivities of blood samples and isolated aortae their blood content was calculated. In control rats infused with saline the blood content was 0.4 μl/100 mg of wet weight. This value was not changed significantly after 6 days' infusion of angiotensin II at three different doses nor in the SHRs (Fig. 4).
In Wistar SHRs of the same age, in which the diastolic blood pressure throughout the 6-day experimental period was higher than that produced by the largest dose of angiotensin, the uptake of LDL or fibrinogen was no greater than, and indeed very similar to, that in the controls.

Taken together, our findings suggest that the effect of angiotensin II in increasing the uptake of LDL and fibrinogen by aortic walls involves blood pressure as one factor, but other factors are also involved.

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