Effect of Angiotensin II and Enalapril on Transfer of Low-Density Lipoprotein Into Aortic Intima in Rabbits

Lars B. Nielsen, Steen Stender, Knud Kjeldsen, Børge G. Nordestgaard

Abstract To assess the mechanism behind a possible atherosclerosis-promoting effect of angiotensin II, the influence of angiotensin II, noradrenaline, and enalapril on transfer of low-density lipoprotein (LDL) into the arterial wall was investigated in conscious rabbits. Intravascular infusion of angiotensin II (1.4 μg/kg per minute) initially increased the mean blood pressure from 70 to 80 mm Hg to 125 to 150 mm Hg; this effect was transient, and the blood pressure returned to baseline values within 2 hours, despite continuous infusion of angiotensin II. The normalized influx of LDL into the aortic intima, determined after in vivo exposure to 125I-LDL for 1 hour, was 88±17 (n=6), 12±12 (n=5), and 28±6 (n=5) nL/cm² per hour (mean±SEM) during angiotensin II infusion at high blood pressure, during angiotensin II infusion after the blood pressure had been normalized, and during continuous saline infusions, respectively (P<.05 for high blood pressure versus low blood pressure and saline). When noradrenaline was used to increase blood pressure to a level similar to that induced by angiotensin II, the normalized influx of LDL in noradrenaline-treated rabbits was also increased markedly. Production of endogenous angiotensin II was inhibited with enalapril (2.9 mg/kg per day). Compared with placebo rabbits, enalapril-treated rabbits had a 92% lower plasma angiotensin-converting enzyme activity and a 23% lower blood pressure. The normalized influx of LDL, however, was similar in the two groups at 18±2 (n=10) and 20±3 (n=10) nL/cm² per hour, respectively. These results suggest that angiotensin II increases the flux of the atherogenic LDL particle from plasma into the arterial wall and that the effect is mediated in large part via increased blood pressure rather than through a direct effect on endothelial permeability. (Circ Res. 1994;75:63-69.)

Key Words • angiotensin II • angiotensin converting enzyme • atherosclerosis • endothelium • lipoprotein

Angiotensin II is a potent vasoconstrictor that can induce high blood pressure. Since hypertension is a risk factor for atherosclerosis and its related diseases, high or low levels of angiotensin II could accelerate or retard the development of atherosclerosis via an effect on blood pressure. Angiotensin-converting enzyme (ACE) inhibitors, which reduce the formation of angiotensin II, protect against atherosclerosis in the Watanabe heritable hyperlipidemic rabbit.1,2 However, the mechanism behind this effect is not clear. Angiotensin II may, in addition to the effect on blood pressure, influence the development of atherosclerosis by a direct effect on vascular cells: the reduction in incidence of myocardial infarction by the ACE inhibitor enalapril in humans could only partly be explained by a lowering of blood pressure.3

The effect of angiotensin II or ACE inhibition on vascular cells has mainly been focused on smooth muscle cells. Angiotensin II stimulated the growth of smooth muscle cells in vitro.4 Similar, in an in vivo rat model of vascular injury, angiotensin II and ACE inhibition induced and reduced smooth muscle cell proliferation, respectively.5,6 In contrast, ACE inhibition failed to affect intimal hyperplasia in injured arteries and vascular grafts of baboons7 and restenosis in patients after percutaneous transluminal coronary angioplasty.8

Other mechanisms may be important in explaining the possible atherogenic role of angiotensin II or an antiatherogenic role of ACE inhibitors, eg, an effect on the transfer of low-density lipoprotein (LDL) across the endothelial barrier into the arterial intima. There is a close correlation between the permeability of a given aortic site to LDL and the subsequent development of atherosclerosis in that site during hypercholesterolemia.9 Angiotensin II inhibited endothelial cell migration in vitro,10 and the ACE inhibitor ramipril protected against the impairment of endothelium-dependent relaxation in cholesterol-fed rabbits.11 On the basis of these observations, it is possible that angiotensin II can interfere with endothelial cell function by a nonpressor mechanism, eg, by increasing the LDL permeability and thereby the transfer of LDL into the arterial wall, with the subsequent accelerated development of atherosclerosis.

The present study investigated to what extent the transfer of LDL from plasma into the arterial wall is affected by exogenous angiotensin II or inhibition of endogenous angiotensin II production by enalapril. To support the hypothesis that angiotensin II increases the normalized influx of LDL via an increased blood pressure, normalized LDL influx was also determined when blood pressure was raised by use of noradrenaline.

Materials and Methods

Animals

Forty-three male white rabbits of the Danish Country Strain (Statens Seruminstitut, Copenhagen, Denmark), weighing 3.0
to 4.0 kg, were maintained under controlled environmental conditions before the in vivo experiments. Sixteen rabbits used to study the effect of angiotensin II and seven rabbits used to study the effect of noradrenaline had free access to a standard rabbit chow (Altromin 1213), whereas 20 rabbits used to study the effect of enalapril received 100 g chow per day: standard rabbit chow was coated with a film (14% ethylcellulose, 28% hydroxymethylcellulose, 54% glucose, and 4% propylene glycol) containing either 10 mg enalapril per 100 g chow (no additive (placebo)). The protocols were approved by the Danish government body supervising animal experiments (Dyreforsøgsstyre).

**Isolation and Labeling of Lipoproteins**

To isolate LDL, human plasma containing Nα-EDETA (final concentration, 1.2 mg/mL), chloramphenicol (80 μg/mL), gentamicin sulfate (80 μg/mL), benzamidin (10 μg/mL), aprotinin (10 kallikrein units/mL), and e-amino-n-caproic acid (2.6 mg/mL) (all from Sigma) was subjected to sequential ultracentrifugation at 4°C in a Beckmann Ti 50.3 rotor for at least 1.8×105 g per minute at solvent densities of 1.019 and 1.063 g/mL, followed by an additional ultracentrifugation washing step at 1.063 g/mL. The protein concentration in the isolated LDL was estimated from the absorbance at 220 nm.

LDL was iodinated by use of iodine monochloride-LDL (0.3 to 1 mL, 5 to 9 mg protein) was mixed with glycine buffer (1 mmol/L, pH 10, 0.4 mL) and 185 to 370 MBq 131I (Amersham) before iodine monochloride was added. The labeled preparations were equilibrated with phosphate-buffered saline containing Nα-EDETA (PBS-EDETA) on a PD-10 column (Sephadex G-25M, Pharmacia), and 100 mg of rabbit albumin was subsequently added. Some labeled preparations were additionally dialyzed overnight against PBS-EDETA. The iodination efficiency was 50±5% (n=12). The specific activity was 0.45 to 1.5×10⁶ cpm/mg protein for 125I-LDL and 0.16 to 0.58×10⁶ cpm/mg protein for 131I-LDL. Of the radioactivity in labeled LDL preparations, 99±0.1% (n=12) was precipitable with trichloroacetic acid (TCA), and 4.5±0.4% (n=12) of the radioactivity was extractable into chloroform/methanol (1:1 vol:voll). After ultracentrifugation of the labeled preparations, 2.4±0.9%, 94.2±1.0%, and 3.4±0.2% (n=12) of the radioactivity had respective density (d) fractions of d<1.019 g/mL, 1.019<d<1.063 g/mL, and d>1.063 g/mL. At 5 minutes, 1 hour, and 3 hours, the respective values after injection of labeled LDL were as follows: 0.6±0.1% (n=27), 1.0±0.2% (n=23), and 1.3±0.2% (n=20) at d<1.019 g/mL; 96.9±0.1%, 95.5±1.1%, and 95.0±0.4% at 1.019<d<1.063 g/mL; and 2.6±0.1%, 3.5±0.3%, and 3.7±0.3% at d>1.063 g/mL. The volume of distribution for the labeled LDL (calculated as the plasma radioactivity concentration 10 minutes after intravenous injection divided by the amount of radioactivity injected and the body weight of the rabbit) was 43±1 mL/kg (n=70 injections of iodinated LDL). No differences between 125I-LDL and 131I-LDL were observed in iodination efficiencies, TCA precipitations and chloroform/methanol extracts, volumes of distribution, and distributions of label between density fractions in labeled preparations and plasma.

For labeling of lipoproteins with [3H]cholesterol ester,5 human plasma was passed through a 0.22-μm filter (Millipore GS, Millipore S.A.) into a rubber-sealed sterile glass container. After addition of (1a,2α)-[3H]-cholesterol (Amersham), the plasma was incubated at 37°C for at least 48 hours, followed by incubation with red blood cells at 4°C to diminish the content of labeled free cholesterol in the lipoproteins by exchange with unlabeled cholesterol in the red blood cells; in the two labeled preparations, 52% and 74% of the 3H radioactivity was in cholesterol esters, as judged by thin-layer chromatography of lipid extracts.

All labeled preparations were passed through 0.22- or 0.45-μm filters (Milliplex GS, Millipore S.A.) before injection.
To determine the importance of plasma contamination, each rabbit was injected with $^{125}$I-LDL 5 to 10 minutes before the aorta was removed. Blood samples were drawn at 10, 30, 60, 120, and 180 minutes. The 3-hour experimental period was chosen to minimize the importance of plasma contamination without introducing too large an error in normalized influx of LDL due to loss of labeled LDL from the aorta. For the aortic arch, it has previously been estimated that calculation of normalized influx of LDL from accumulation of labeled LDL during 3 hours leads to an underestimation of normalized influx by 7% to 8%. In the thoracic and abdominal aorta, this underestimation was less than 33% to 69%.\(^9\) This implies that normalized influx values for the thoracic and abdominal aorta based on 3-hour LDL accumulation should be interpreted with caution.

All rabbits were killed by an intravenous injection of pentobarbital (40 to 80 mg/kg body wt). A needle was inserted into the left ventricle of the heart, and the systemic circulation was perfused with 800 to 1000 mL of PBS (4°C) before the aorta was removed.

**Analysis**

Immediately after its removal, the aorta was freed of adventitia and opened longitudinally. The area of the intimal surface was outlined on graph paper before the aorta was divided into its arch, thoracic aorta, and abdominal aorta at the level of the first intercostal branches and celiac axis, respectively. In the angiotensin II and in the noradrenaline experiment, each of these three aortic segments was subdivided into a proximal and a distal segment of similar size.

The intima/inner media of each aortic segment was stripped from the outer media, and the intima/inner media was minced with scissors in 900 μL cold saline with 100 μL human albumin solution (100 mg/mL) or 100 μL human plasma added (to provide protein mass for subsequent protein precipitations and adding to the cholesterol ester mass as a carrier for $^{131}$I-cholesterol ester during thin-layer chromatography procedures). Proteins were precipitated with TCA at a final concentration of 15% (wt/vol) at 4°C. Similarly, aliquots of plasma samples (10 to 25 μL) and diluted doses to which 900 μL cold saline and 100 μL albumin solution (or human plasma) had been added were also TCA-precipitated. After mixing and centrifugation, total and TCA-soluble radioactivity in the supernatant was determined by counting samples in a double-channel gamma counter (Selektronik). $^{131}$I and $^{31}$I were determined after corrections for spillover, background, and decay of $^{131}$I. All samples were counted for 42 to 60 minutes; maximal standard deviations for observed counting rates of $^{125}$I and $^{31}$I in TCA precipitates of arterial samples were <2%. Typical standard deviations were <1% for both isotopes.

Lipids in the TCA precipitates of aortic intima/inner media and aliquots of plasma and doses were extracted for at least 24 hours with chloroform/methanol (2:1 [vol/vol]), after addition of methanol to form a 1:1 chloroform/methanol solution, precipitates were centrifuged and washed twice with chloroform/methanol (1:1). Esterified cholesterol was isolated from the extract by thin-layer chromatography,\(^{15}\) and the amount of $^3$H was determined by liquid scintillation counting for 60 minutes in a TRICARB 2000 counter (Packard). The maximal standard deviation for counting rates of $^3$H in aortic-esterified cholesterol was 3.1%; no $^{131}$I or $^{31}$I was detected in the esterified cholesterol fraction.

Fixed-density ultracentrifugations were performed at 4°C in a Beckmann 50.3 T) rotor for at least 1.8×10⁶g/min per minute (average)\(^9\) after adjusting plasma and dose material with added carrier plasma to densities of 1.019 and 1.063 g/mL by addition of Na₂EDTA containing NaBr solutions. After tube slicing, radioactivity and cholesterol were determined in top and bottom fractions. Plasma and lipoprotein cholesterol concentrations were determined with the CHOD-PAP enzymatic method (Boehringer Mannheim).

**Calculations**

TCA-precipitable radioactivity in tissues, plasma, and doses was used in the calculations. Since plasma macromolecules probably enter the aortic media from both the luminal and adventitial side and since radioactivity in the intima is considered to be derived primarily from the lumen of the aorta,\(^20,21\) only radioactivity in the intima/inner media was included in the calculations.

Since the flux of LDL into aorta is proportional to the plasma LDL concentration,\(^22\) the influx of LDL in each rabbit was normalized to the same plasma LDL concentration and expressed in plasma equivalents. The normalized influx of LDL (I, in nanoliters per square centimeter per hour) can be calculated from the aortic radioactivity at the end of the experiment (A, in counts per minute per square centimeter), the mean plasma radioactivity concentration (C₉₀₀, in counts per minute per nanoliter), and the length of the experiment (t, in hours) as follows:

\[
I = \frac{A}{C_{900} \times t}
\]

Before calculation, the fraction of aortic radioactivity estimated to be due to plasma contamination was subtracted. The plasma contamination (in nanoliters per square centimeter) was determined by dividing the amount of $[^3]$Hcholesterol ester (angiotensin II experiment: rabbits infused with saline from 0 to 120 minutes and angiotensin II from 120 to 180 minutes) or $^{125}$I (noradrenaline and enalapril experiment) in the arterial tissue by the respective plasma radioactivity concentration at the end of the experiment (in counts per minute per nanoliter). Those values for plasma contamination were then multiplied by the final plasma radioactivity concentration of the isotope used to measure normalized influx of LDL (i.e., $^{125}$I in the angiotensin II experiment and $^{131}$I in the noradrenaline and enalapril experiment). This value was subtracted from the aortic radioactivity of this second isotope before calculation of the normalized influx of LDL using Equation 1. In the angiotensin II experiment, the normalized LDL influx values of rabbits infused with angiotensin II from 0 to 180 minutes and rabbits infused with saline from 0 to 180 minutes were corrected for plasma contamination by using an average plasma contamination value of 30 nL/cm² per hour.

**Plasma Contamination**

The average plasma contamination in the aortic arch, the thoracic aorta, and the abdominal aorta combined was 25±3 nL/cm² (n=27 tissues) in rabbits with high blood pressure (angiotensin II-infused and noradrenaline-infused rabbits combined) and 31±2 nL/cm² (n=72 tissues) in rabbits with normal or low blood pressure (saline-infused rabbits in noradrenaline experiment and all rabbits in the enalapril experiment combined). In the noradrenaline experiment, the plasma contamination was 30±4, 19±3, 16±2, 24±4, 35±6, and 28±2 nL/cm² (n=7 rabbits) in aortic segments 1, 2, 3, 4, 5, and 6, respectively.

Since plasma contamination is determined from aortic intimal/medial radioactivity content after exposure to labeled LDL for 5 to 10 minutes, contamination values presumably reflect the sum of radioactivity in plasma adhering to the arterial surface and labeled LDL that has entered the arterial wall during the exposure period.\(^21\) The similar plasma contamination in rabbits with high blood pressure compared with rabbits with normal blood pressure, in spite of a several-fold increase in LDL influx in rabbits with high pressure, supports the notion that labeled LDL that has entered the arterial wall plays a minor role in determining the plasma contamination.

Plasma contamination correction had no influence on the observed difference in LDL influx between hypertensive and normotensive rabbits. Thus, whether normal or high LDL influx is calculated with or without plasma contamination corrections, similar conclusions will be drawn from the present results.
The present average plasma contamination value is two to five times higher than in previous reports. No technical explanation can be provided for this observation. The plasma contamination was similar when determined with iodinated LDL, lipoproteins labeled in vitro with $[^3H]$cholesterol ester, or lipoproteins labeled in vivo by oral administration of $[^3H]$cholesterol to a cholesterol-fed rabbit; the latter results were obtained in four additional rabbits (data not shown).

**Statistics**

Values are presented as mean±SEM. All P values are two tailed. Statistical analyses of differences between mean values were based on Student’s t tests. Mean normalized LDL influx values in the angiotensin II experiment, however, were compared by a one-way ANOVA using Bonferroni’s adjustment for multiple comparisons.

**Results**

**Effect of Angiotensin II**

Intravascular infusion of angiotensin II (1.4 μg/kg per minute) into 11 conscious rabbits had no visible side effects. The mean blood pressure was initially increased markedly but returned to baseline values within 2 hours of continuous angiotensin II infusion (Fig 1, middle panel). The normalized flux of LDL into the intima of the aortic arch in angiotensin II–treated rabbits with elevated blood pressure was higher than in saline-infused control rabbits ($P<.05$) and in angiotensin II–treated rabbits when blood pressure had returned to baseline values ($P<.05$) (Fig 2). There was no statistically significant difference between saline-infused rabbits and angiotensin II–treated rabbits when blood pressures had returned to baseline values.

Fig 3 depicts the normalized influx of LDL in six different aortic sites in saline-infused control rabbits with normal blood pressure and in angiotensin II–treated rabbits with high blood pressure. In both groups, the normalized influx of LDL decreased throughout the length of the aorta; the values for angiotensin II–treated rabbits when blood pressures had returned to normal values showed a similar regional distribution (data not shown). In all aortic sites except for the most distal abdominal segment, normalized influx of LDL appeared higher in angiotensin II–treated rabbits with high blood pressure than in saline-infused control rabbits.

**Effect of Noradrenaline**

Infusion of noradrenaline (6 to 17 μg/kg per minute) had no visible side effects in three rabbits and increased mean blood pressure to an average of 110±5 mm Hg (Fig 4). This increased blood pressure was similar to the average mean blood pressure during the first hour in rabbits infused with angiotensin II (1.4 μg/kg per minute). The mean blood pressure was an average 69±4 mm Hg in four saline-infused rabbits. Similar to the results of the angiotensin II experiment, the normalized influx of LDL decreased throughout the length of the aorta and was higher in the hypertensive noradrenaline-infused rabbits than in normotensive saline-infused control rabbits (Fig 4).
Effect of Enalapril

Oral enalapril treatment (2.9 mg/kg per day) during 2 weeks had no visible side effects. All rabbits ate their daily allowance, and the plasma enalaprilat concentration increased to 79±10 ng/mL. Plasma ACE activity was reduced by 92% and mean blood pressure by 23% in enalapril-treated rabbits compared with placebo-treated rabbits (Table). However, there was no difference in normalized influx of LDL into the aorta between the two groups (Table). Plasma LDL cholesterol levels were similar in enalapril- and placebo-treated rabbits (0.43±0.10 and 0.50±0.08 mmol/L, respectively), and there was no difference in total plasma, very-low-density lipoprotein, or high-density lipoprotein cholesterol concentrations (data not shown).

Effect of Enalapril (2.9 mg/kg per day) on Angiotensin-Converting Enzyme Activity, Blood Pressure, and Normalized Flux of Low-Density Lipoprotein Into Aortic Intima

<table>
<thead>
<tr>
<th></th>
<th>Enalapril (n=10)</th>
<th>Placebo (n=10)</th>
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<tbody>
<tr>
<td>ACE activity, U/L</td>
<td>7±1*</td>
<td>88±4</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
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<tr>
<td>Systolic</td>
<td>70±2*</td>
<td>81±3</td>
</tr>
<tr>
<td>Mean</td>
<td>53±2*</td>
<td>69±2</td>
</tr>
<tr>
<td>Diastolic</td>
<td>45±2*</td>
<td>59±2</td>
</tr>
<tr>
<td>Normalized influx of LDL, nL/cm² per hour</td>
<td></td>
<td></td>
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<tr>
<td>Aortic arch</td>
<td>18±2</td>
<td>20±3</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>6±2</td>
<td>4±1</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>7±1</td>
<td>4±2</td>
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</tbody>
</table>

ACE indicates angiotensin-converting enzyme; LDL, low-density lipoprotein. Values are mean±SEM.

Rabbits were treated with enalapril for 2 weeks before determination of normalized influx of LDL. ACE activity was determined after 1 and after 2 weeks; the data are mean values of the two measurements. Blood pressure was determined after 1 week.

*P<.001 compared with the placebo group.

Discussion

Effect of Angiotensin II on Transfer of LDL Into Aorta

The marked increase in blood pressure induced by angiotensin II infusion (1.4 μg/kg per minute) in conscious rabbits was transient: blood pressure returned to baseline values 1 to 2 hours after start of the infusion. Gavras et al.28 showed that continuous infusion of angiotensin II in rabbits at 0.9 to 1.8 μg/kg per minute increased plasma angiotensin II from 0.2±0.06 ng/mL (mean±SEM, n=5) to 11.2±7.6 ng/mL after 6 hours. The same authors reported angiotensin II levels as high as 5 ng/mL in patients with malignant hypertension. This suggests that infusion of angiotensin II at 1.4 μg/kg per minute in the present experiment induced marked increases in plasma levels of angiotensin II and that these plasma levels may be slightly higher than those in humans with malignant hypertension.

The temporary effect of angiotensin II on blood pressure allowed assessment of the effect of angiotensin II on the normalized influx of LDL independent of the effect on blood pressure: the normalized influx of LDL was increased by angiotensin II only when blood pressure was elevated. The observation suggests that the increase in transfer of LDL across the endothelium by angiotensin II is secondary to the effect on blood pressure. It cannot be excluded, though, that a possible direct effect of angiotensin II on endothelial permeability diminished coincidently with the temporary effect on blood pressure, eg, through tachyphylaxis or production of antagonists to angiotensin II. However, when noradrenaline was used to increase blood pressure to a level similar to that induced by angiotensin II, the normalized influx of LDL in noradrenaline-treated rabbits was increased to at least the same extent as in angiotensin II–treated rabbits. This supports the idea that a pressure effect is the major determinant for the increased transfer of LDL into the aorta during the first hour of angiotensin II infusion rather than a direct effect of angiotensin II on endothelial permeability.

Finally, enalapril did not affect the normalized influx of LDL, in spite of a 92% reduction in plasma ACE activity. These results render a direct effect of endogenous angiotensin II on endothelial permeability to LDL unlikely under the present experimental conditions.

The present findings contrast with those of a previous study16 reporting that angiotensin II increased the aortic uptake of albumin in rabbits independent of blood pressure. One major difference between the two studies is that the former study used anesthetized rabbits, whereas the present one used conscious rabbits. Also, the apparent discrepancy between the results of the two studies could be related to possible differences in the transfer of LDL and albumin into the arterial wall: LDL is a larger particle with a diameter of ≈25 nm compared with an 8-nm diameter for albumin.

To assess the effect of angiotensin II on the transfer of LDL into the arterial wall, the normalized influx of LDL was determined from the accumulation of labeled LDL during 1 hour. This short uptake time indicated that the amount of labeled LDL entering the arterial intima/inner media was comparable to the amount of labeled LDL adhering to the endothelial surface after a 5- to 10-minute exposure (referred to as plasma con-
tamination). The plasma contamination was similar in rabbits with high blood pressure and in normotensive rabbits in spite of several-fold differences in normalized LDL influx. Consequently, similar results and the same conclusions were obtained both with and without adjusting the aortic radioactivity for plasma contamination before calculations of normalized LDL influx values.

**Effect of Blood Pressure on Transfer of LDL Into Aorta**

It has long been recognized that high blood pressure increases the transfer of plasma proteins such as albumin across the endothelium. Less information is available on the effect of hypertension on the transport of LDL into the arterial wall. Data from Bretherton et al suggest that chronic hypertension induces alterations in the endothelium affecting the barrier function toward LDL: entry of iiodinated LDL into the aortic intima was increased in rabbits that had been hypertensive for >4 weeks, and this effect was maintained when the blood pressure was acutely normalized. It is also possible, though, that these results at least partially reflect an increased distribution volume for labeled LDL in arteries exposed to hypertension.

Considering the effect of an acute increase in blood pressure, Fry et al found no effect of pressure on the intimal/medial uptake of iodinated LDL in excised pig aortas with intact endothelium but a significant effect of pressure on LDL uptake if the endothelium was removed. On the other hand, high pressure has been shown to increase the uptake of iodinated LDL in freshly excised rabbit aortas. The present study further supports the notion that acute increases in blood pressure, induced by angiotensin II or noradrenaline, can induce increases in transendothelial transport of LDL in the rabbit aorta in vivo.

To the best of our knowledge, no reports have evaluated the effect of subnormal blood pressure on the endothelial barrier function in vivo. In normotensive rabbits, mean blood pressure was lowered by an average of 16 mm Hg after 1 week of treatment with enalapril. However, enalapril did not significantly affect the normalized flux of LDL into the aorta. This was in contrast to the marked effect on normalized LDL influx when blood pressure was acutely increased by an average of 25 mm Hg in the angiotensin II experiment and 41 mm Hg in the noradrenaline experiment. Since blood pressure was determined 1 week before and not at the time of the influx determinations in the enalapril experiment, we cannot exclude the possibility that blood pressure returned toward normal values during the second week of enalapril treatment, although ACE activity remained depressed by enalapril. Given this reservation, the present data are compatible with the presence of a threshold for the effect of blood pressure on transport of LDL into the aorta intima.

Some investigators have suggested that high blood pressure may have different effects on transendothelial transport of plasma macromolecules at different aortic sites. In the present study, the use of contamination-corrected normalized LDL influx values illustrated a considerable variation along the length of aorta, which is in close agreement with previous results. This regional variation was preserved even at high blood pressures in both the angiotensin II and the noradrenalin experiments. Combining these two experiments, the level of normalized LDL influx appeared higher in all aortic sites in hypertensive compared with normotensive rabbits. Considering the relatively low statistical power in the present study, the results suggest that high blood pressure has a similar effect on the transfer of LDL across the endothelium throughout the length of the aorta.

To understand mechanistically the effect of high blood pressure on the transport of LDL across the endothelial layer, macromolecular pathways across the endothelium have to be considered. Some ultrastructural observations favor a vesicular transport of LDL, and an increasing number of vesicles in endothelial cell have been observed during hypertension in the rat. Others have suggested that transfer of plasma macromolecules occurs mainly through interendothelial pores/gaps and that angiotensin II may increase the size of such pores/gaps. The nature of pores in the endothelium that allow passage of LDL with a diameter of >20 nm, however, has not been clearly described on the ultrastructural level. Weinbaum et al have provided a model for macromolecular transport in relation to leaky intercellular junctions, which predicts that even though such open junctions occupy a fraction of <1×10⁻⁵ of the en face area of the endothelial surface, they may account for the bulk transendothelial transport of plasma macromolecules. Such junctions may easily be overseen in conventional morphological examination of the endothelium. Lin et al have linked the turnover of single endothelial cells to leaking of plasma macromolecules into the arterial wall, and the same group has shown that chronically hypertensive rats have more such leaky junctions than do normotensive rats. The presence of open junctions in the endothelium at high blood pressure would explain an increased normalized influx of LDL by an increased convective transport of LDL, as predicted in a model by Fry on transport of plasma macromolecules into the arterial wall.

**The Renin-Angiotensin System and Atherogenesis**

In addition to the effect mediated via blood pressure, the renin-angiotensin system may affect the development of atherosclerosis by a direct effect on the arterial wall. High plasma levels of renin as well as the DD genotype of the insertion/deletion polymorphism in the ACE gene associated with high plasma ACE levels are associated with an increased risk for myocardial infarction, independent of the blood pressure. The mechanism behind these findings is not clear.

Transfer of the atherogenic LDL particle into the arterial intima with subsequent deposition of cholesterol is a key phenomenon in atherogenesis. The present study could not demonstrate a direct effect on the barrier function of the intact nonatherosclerotic arterial wall by the renin-angiotensin system, as approached by angiotensin II infusion or ACE inhibition. However, it is certainly possible that angiotensin II affects other variables related to the kinetics of atherogenic lipoproteins in the arterial wall, such as efflux and degradation. Also, a study by Chang et al suggested that LDL accumulates preferentially at aortic sites of endothelial regeneration after mechanical damage to the endothelium. It is possible that the putative atherogenic effect of angiotensin II occurs at such sites, since angiotensin II inhibits the migration of
endothelial cells in vitro. Still, transfer of LDL into the arterial intima was increased at supranormal blood pressure induced by angiotensin II or noradrenaline. This supports the notion that ACE inhibition in hypertension protects the intact arterial wall against atherosclerosis through its lowering of the blood pressure, resulting in reduced influx of atherogenic lipoproteins into the arterial intima.

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