Hypoalbuminaemia enhances the renal vasoconstrictor effect of lysophosphatidylcholine

Thi Danh Vuong, Branko Braam, Nel Willekes-Koolschijn, Peter Boer, Hein A. Koomans and Jaap A. Joles

Department of Nephrology and Hypertension, University Medical Center, Utrecht, The Netherlands

Abstract

Background. Lysophosphatidylcholine (LPC) causes vascular dysfunction in vitro. Lipoprotein LPC is increased in hypoalbuminaemia. Albumin binds LPC and restores LPC-induced abnormalities. We hypothesized that in vivo LPC impairs blood flow more in hypoalbuminaemia than in normoalbuminaemia.

Methods. Increasing concentrations of LPC were infused intra-renally in Nagase analbuminaemic rats (NAR) and Sprague–Dawley rats (controls).

Results. Intra-renal LPC (0.1 μmol/min, 20 min) reduced renal blood flow (RBF) more (P < 0.01) in NAR (from 8.3 ± 0.3 to 4.0 ± 1.1) than in controls (from 7.7 ± 0.7 to 5.8 ± 0.5 ml/min/g kidney). Lysophosphatidylethanolamine had no effect. After stopping LPC, RBF recovery was delayed in NAR [median 90 (range: 70–90) vs 45 min (40–60), P < 0.01]. Intravenous bovine serum albumin (BSA) prevented LPC-induced vasoconstriction in both strains. Prolonging LPC for 60 min delayed recovery of RBF. In this setting, intra-renal BSA completely restored RBF in 75 min (30–90), while intra-renal saline only resulted in 33±13% recovery (P < 0.01). Baseline renal LPC content was unchanged in NAR. However, intra-renal LPC infusion doubled renal LPC content in NAR, but had no effect in controls.

Conclusions. In NAR, baseline RBF and renal LPC content are normal. However, exposure of NAR to LPC results in much more vasoconstriction and accumulation of LPC than in normoalbuminaemia. Addition of albumin prevents and restores LPC-induced vasoconstriction.

Keywords: albumin; analbuminaemic rat; lysophosphatidylcholine; hypoalbuminaemia; renal blood flow

Introduction

Endothelial cells are continually exposed to circulating lipids and, in some circumstances, to lipids that have accumulated in subendothelial regions. The oxidative products formed by cells of the artery wall can react with low-density lipoprotein (LDL) trapped in the subendothelial space and initiate lipid oxidation. Oxidized LDL (ox-LDL) has been shown to cause contraction and to impair endothelium-dependent relaxation (EDR) [1,2]. This may directly contribute to the increased risk of vasospasm seen in hypercholesterolaemia and atherosclerosis. This inhibition is specific for ox-LDL as comparable concentrations of native LDL do not have this effect [1,2].

Impairment in EDR induced by ox-LDL cholesterol in vitro could be ascribed to lysophosphatidylcholine (LPC), a component formed during oxidative modification of LDL cholesterol [3]. Under normal circumstances LPC is tightly bound to plasma albumin [4]. Indeed, in vitro, LPC-induced impairment of EDR was restored by albumin [5]. In conditions where the plasma albumin level is low, as in nephrotic syndrome, most of the LPC normally bound to albumin is shifted to lipoprotein [6]. In subjects with hypoalbuminaemia in combination with proteinuria, LPC shifts from albumin to LDL, an effect independent of hyperlipidaemia [7]. Increased levels of LPC in the LDL fraction have been found in both hypoalbuminaemic nephrotic rats and non-proteinuric Nagase analbuminaemic rats (NAR) [6]. In NAR, LPC levels are also increased in erythrocyte membrane accompanied by a strong decrease in erythrocyte deformability and increased whole blood viscosity. Albumin normalized LPC content in NAR red cell membranes and restored erythrocyte deformability [8]. However, in the absence of albumin other cell membranes besides those of erythrocytes will also be exposed to high levels of LPC.

Recently, we found markedly protective effects of albumin on LPC-induced attenuation of vasodilatation
in vitro [5]. Moreover, phenylephrine-induced vasoconstriction was also enhanced by LPC, and this was also reversed by albumin. However, the importance of albumin on LPC-induced vasoconstriction in vivo is unknown. Therefore, the present study addressed the question whether infusion of LPC, in vivo, results in more profound renal vasoconstriction in analbuminemia. Moreover, we questioned whether LPC-induced vasoconstriction would persist for a longer period if albumin were absent. To answer this hypothesis, the vasoconstrictive effect of LPC on the renal blood flow (RBF) and subsequent recovery, was studied in NAR and control Sprague–Dawley rats. In addition, renal LPC content was measured under baseline conditions, and directly after intra-renal LPC administration. Experiments were also conducted to study whether albumin can prevent and restore the LPC-induced decrease in RBF in NAR.

Subjects and methods

Animal preparation

Male Sprague–Dawley (200–350 g; Harlan-Olac, The Netherlands) and NAR (200–350 g; GDL, The Netherlands) were housed in a temperature- and light-controlled room. The NAR consistently have albumin levels <0.3 g/l[6,8]. The rats had free access to standard rat chow (Hope Farms, Woerden, The Netherlands) and tap water. Sentinel animals were monitored regularly for infection by nematodes and pathogenic bacteria, as well as antibodies for a large number of rodent viral pathogens, and were consistently negative throughout the course of the experiments. The Utrecht University board for studies in experimental animals approved the studies.

On the day of the experiment, the animals were anaesthetized with sodium pentobarbital (60 mg/kg body weight, i.p.) and placed on a thermo-regulated surgical table that maintained rectal temperature at 37°C. After tracheotomy, the renal artery was cannulated with a polyethylene catheter (tapered PE-10), via the carotid artery, for the infusion of intra-renal solutions. All intra-renal infusions were passed through a Millipore filter (5 μm, Schleicher and Schuell, Dassel, Germany). The left jugular vein was catheterized (PE-50) for infusion of polyfructosan in saline, in some cases with bovine serum albumin (BSA) (see below). Before surgery the PE-50 catheter was fitted with a thin catheter (PE-10) for anaesthetic administration of sodium pentobarbital (6 mg/kg/h). A catheter (PE-50) was inserted via the left femoral artery to measure arterial pressure (MAP) using a pressure transducer (Transpac IV, Abbott, Ireland) and to collect blood samples. The bladder was catheterized using a flanged (PE-50) catheter for collection of right kidney urine samples. The left kidney was approached by a flank incision, freed from surrounding tissue and placed in a plastic holder; the left urether was cannulated with a PE-10 catheter. A 1R8 ultrasonic flow probe was placed around the left renal artery and connected to a transit time blood flow meter (model T206; Transsonic System Inc., Ithaca, NY) for the measurement of RBF.

All animals received intravenous (i.v.) and intra-renal infusions of a 150 mmol/l NaCl solution throughout the experiments. The i.v. infusion, which also contained 15% polyfructosan (Inutex, Fresenius Pharma, Austria), was at a rate of 10 μl/min/100 g body weight, and the intra-renal infusion at a rate of 10 μl/min. A 60-min equilibration period was observed before the start of the measurements. At the end of each experiment blood was drawn from the renal vein with a syringe, and kidneys were removed, blotted dry and weighed. To assess glomerular filtration rate (GFR) inulin in plasma and urine was measured photometrically with indolacetic acid after hydrolysis to fructose. Urine sodium was measured with flame photometry.

Renal phospholipids

Renal LPC, sphingomyelin (SM) and phosphatidylycholine (PC) content were measured by normal phase HPLC [9]. Kidneys were stored at −80°C until use and they were homogenized in 3.3 ml 0.9% NaCl/g tissue. To 0.5 ml of the homogenates 2.5 ml methanol was added, and after vortexing and 15 min waiting, 2.5 ml chloroform and 50 μl 0.5% butylated hydroxytoluene were added. The mixture was vortexed and centrifuged, and the organic phase was collected. The extraction was repeated once and the combined extracts were washed with 5 ml 1% NaCl. After centrifugation, the organic phase was collected and dried with a stream of N2. Residues were dissolved in 100 μl HPLC eluent A (see below) and stored at −20°C.

HPLC was performed with a Merck-Hitachi L-6200A and L-6000 dual pump system (Merck, B.V., Amsterdam, The Netherlands), equipped with a Rheodyne injector and a 100 × 4.6 mm Allsphere 3 μm Silica column (Alltech, Breda, The Netherlands). For signal detection and quantification of LPC, SM and PC an Alltech ELSD2000 evaporative light scattering detector was used operated at 70°C at a nitrogen flow rate of 2 l/min. As eluents the following isopropanol–hexane–water–ammonia mixtures were used: eluent A 67.8:40:2:0.2 and eluent B 51.8:40:8:0.2 (by volume). Mobile phase flow rate was 1.25 ml/min, column oven temperature 30°C, and injection volume 10 μl. Elution was performed with a linear gradient from 100% A to 100% B in 20 min, followed by 100% B for 20 min. Typically, LPC eluted at 26.4 min, SM at 21.9 and 22.4 min (double peak), PC at 20.8 min, and other phospholipids (not quantified) at <15 min. The assay coefficients of variation of LPC, SM, and PC were 8, 9 and 6%, respectively.

Experimental design (Figure 1)

There were four separate studies. In the first and fourth study recovery of RBF was followed after discontinuation of the lysophospholipid infusion.

The first study assessed the dose–response to LPC in both Sprague–Dawley rats (n = 6) and NAR (n = 7) or to lysophosphatidylethanolamine (LPE) in NAR (n = 3). LPC or LPE [both containing primarily palmitic (C16:0) and stearic (C18:0) acids, Sigma, St Louis, MO] were dispersed in saline by ultrasound and diluted from 10 to 0.001 mmol/l. Increasing concentrations of LPC or LPE were given intra-really at a rate of 10 μl/min, and maintained for 20 min for each concentration. Thus, at the highest concentration the amount of LPC infused was 0.1 μmol/min. Because RBF was close to 10 ml/min in both strains (see Results), we estimate renal LPC and LPE concentrations in the blood to be
performed as in the first study. Blood samples were taken at an interval of 60 min. Terminal bovine albumin concentrations were determined in renal vein and femoral arterial plasma samples.

Calculations and statistics

Renal vascular resistance (RVR) was calculated as MAP divided by RBF corrected for kidney weight and given in units. In the experiments, which were done to determine the dose–response effect of LPC on RBF, the average MAP and RBF were taken in the last 10 min of each period. In the experiments where only 10 mmol/l LPC was used, averages were taken in the last 20 min. Recovery time was defined as the time, after stopping LPC, needed for RBF to return to at least 95% of baseline. Data presented in the tables are results from the last period of every infusion. Data were compared with two-way analysis of variance for repeated measurements. If a variance ratio reached statistical significance, the Student Newman–Keuls test was performed as a post-hoc test. Recovery times were compared non-parametrically with the Welch test. A value of $P < 0.05$ was considered significant.

Results

Animal data

Body weights of Sprague–Dawley rats (284 ± 14 g) and NAR (283 ± 9 g) were comparable. Kidneys were heavier in Sprague–Dawley rats (1.10 ± 0.04 g) than in NAR (0.95 ± 0.02 g) ($P < 0.05$).

Effect of intra-renal infusion of LPC on renal haemodynamics

There were no differences in MAP between Sprague–Dawley rats ($n = 6$) and NAR ($n = 7$) at baseline (saline) (Table 1). Intra-renal infusion of increasing concentrations of LPC, with a total duration of 100 min, had no effect on blood pressure, neither in Sprague–Dawley rats nor in NAR, but gave a dose-dependent decrease in RBF that was more pronounced in the NAR (Figure 2). This figure also demonstrates that RBF in the NAR was already significantly decreased with infusion of 3 mmol/l LPC ($P < 0.05$), while no effect was seen in the Sprague–Dawley rats at this concentration. Infusion of the highest dose of LPC (10 mmol/l) significantly decreased in RBF observed in both strains. However, this change in RBF was significantly less in Sprague–Dawley rats than in NAR (RBF = 1.8 ± 0.4 and 4.7 ± 0.7 ml/min/g kidney weight, respectively, $P < 0.01$). Note that vasoconstriction persisted in the first recovery period, suggesting continued LPC infusion in part of this period, because of catheter dead-space. RVR increased significantly after the highest dose of LPC, and the change in RVR was also greater in the NAR (Sprague–Dawley rats: RVR = 5.3 ± 1.7 U and NAR: RVR = 19.9 ± 5.4 U, $P < 0.01$). Changes induced by the highest LPC dose are listed in Table 1. Changes in urine flow, GFR and natriuresis of the left kidney were
parallel to RBF. LPC infusion in the left kidney had no effects on renal haemodynamics in the right kidney (data not shown). The time needed for recovery of RBF (i.e. 95% of mean baseline) after stopping LPC in Sprague–Dawley rats and NAR was 45 (range: 40–60) vs 90 min (70–90), respectively \( (P < 0.01; \text{Figure 2}). \)

### Effect of intra-renal infusion of LPC on renal phospholipids

Baseline renal LPC content was not different in NAR and Sprague–Dawley rats (Figure 3). However, intra-renal LPC administration more than doubled renal LPC content in NAR \( (P < 0.05 \text{ vs all other groups}) \), whereas there was no change in Sprague–Dawley rats. There were no differences in renal SM and PC content in any of the groups (data not shown).

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### Table 1. Renal function in Sprague–Dawley rats \((n = 6)\) and NAR \((n = 7 \text{ for LPC}; n = 3 \text{ for LPE})\) during intra-renal saline (baseline) and the highest LPC or LPE concentration (10mmol/l)

<table>
<thead>
<tr>
<th></th>
<th>Sprague–Dawley saline</th>
<th>Sprague–Dawley + LPC</th>
<th>NAR saline before LPC</th>
<th>NAR + LPC</th>
<th>NAR saline before LPE</th>
<th>NAR + LPE</th>
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<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>121 ± 1</td>
<td>117 ± 3</td>
<td>120 ± 2</td>
<td>117 ± 1</td>
<td>118 ± 2</td>
<td>116 ± 2</td>
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<tr>
<td>Haematocrit (%)</td>
<td>48 ± 2</td>
<td>46 ± 3</td>
<td>48 ± 0</td>
<td>46 ± 1</td>
<td>47 ± 1</td>
<td>45 ± 1</td>
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<tr>
<td>RBF (ml/min/g kidney)</td>
<td>7.7 ± 1.8</td>
<td>5.8 ± 0.5(^*)</td>
<td>8.3 ± 0.3</td>
<td>4.0 ± 1.1(^*)</td>
<td>8.1 ± 0.7</td>
<td>8.0 ± 0.7</td>
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<tr>
<td>RVR (U)</td>
<td>14.9 ± 1.1</td>
<td>21.0 ± 2.8(^*)</td>
<td>14.6 ± 0.4</td>
<td>34.4 ± 5.2(^*)</td>
<td>14.8 ± 1.4</td>
<td>14.4 ± 1.3</td>
</tr>
<tr>
<td>Urine flow (ml/min)</td>
<td>8.2 ± 1.1</td>
<td>5.3 ± 1.1(^*)</td>
<td>7.6 ± 0.5</td>
<td>2.9 ± 1.0(^*)</td>
<td>7.7 ± 1.3</td>
<td>9.3 ± 3.1</td>
</tr>
<tr>
<td>GFR (ml/min/g kidney)</td>
<td>1.08 ± 0.15</td>
<td>0.51 ± 0.19(^*)</td>
<td>1.03 ± 0.04</td>
<td>0.53 ± 0.01(^*)</td>
<td>0.99 ± 0.09</td>
<td>1.02 ± 0.10</td>
</tr>
<tr>
<td>Natriuresis (μmol/min)</td>
<td>1.1 ± 0.5</td>
<td>0.3 ± 0.2(^*)</td>
<td>1.0 ± 0.2</td>
<td>0.2 ± 0.1(^*)</td>
<td>1.0 ± 0.6</td>
<td>1.2 ± 0.9</td>
</tr>
</tbody>
</table>

\(^*P < 0.05 \text{ vs saline.}\)

\(^\text{b}P < 0.01 \text{ vs Sprague–Dawley + LPC.}\)

The last 10-min LPC or LPE (10mmol/l) period was compared with the last 10 min of the baseline period.

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### Effect of intra-renal infusion of LPE on renal haemodynamics

There were no differences in MAP before and after intra-renal LPE infusion in the NAR \((n = 3)\) or compared with baseline MAP in NAR infused with LPC (Table 1). Intra-renal infusion of increasing concentrations of LPE had no significant effects on blood pressure, RBF, RVR, urine flow, GFR or natriuresis (Table 1).
I.v. infusion of albumin prevents LPC-induced renal vasoconstriction

I.v. BSA resulted in BSA concentrations of 26±3g/l in the Sprague-Dawley rats and 21±1g/l in the NAR. Under these circumstances LPC-induced renal vasoconstriction was prevented (Sprague-Dawley: 10.4±0.4 to 10.4±0.6, NAR: 11.2±0.9 to 9.1±1.0 ml/min/g kidney). However, this manoeuvre also caused considerable volume expansion judged by the fall in haematocrit (Sprague-Dawley: 48±2 to 40±1, NAR: 46±1 to 37±1%) and enhanced diuresis (Sprague-Dawley: 14±2 to 26±4, NAR 13±5 to 22±7 μl/min) and natriuresis (Sprague-Dawley: 2.0±0.4 to 4.6±1.1, NAR: 1.5±0.3 to 3.1±1.1 μmol/min).

Intra-renal infusion of albumin restores LPC-induced renal vasoconstriction

Because the vasoconstriction achieved by LPC was stronger in NAR than in Sprague-Dawley rats, it was difficult to judge whether the more rapid recovery of Sprague-Dawley rats was due to albumin. Therefore, to study the effect of albumin on LPC-induced renal vasoconstriction, we infused LPC in the maximum dosage used in the first protocol, i.e. 10 mmol/l for 60 min in two groups of NAR, followed either by intra-renal 4% BSA or intra-renal saline. Changes induced at the end of the infusion period are listed in Table 2. Again, LPC caused a significant reduction in RBF, accompanied by a significant increase in RVR (P < 0.01, LPC vs baseline) (Table 2). Compared with the first protocol, where 10 mmol/l LPC was infused for only 20 min, the recovery of RBF was delayed [90 min (range: 70–90) after stopping LPC 95% recovery had been achieved in the first protocol as compared with only 42±8% in the second protocol in the same period; P < 0.05]. Intra-renal infusion of 4% BSA, completely restored RBF in for 75 min (30–90); at this time point spontaneous recovery was only 33±13% (P < 0.05; Figure 4). The renal venous and femoral arterial plasma concentration of BSA obtained at the end of the experiments were 6.3±0.6 and 2.5±0.8 g/l, respectively (n = 6). MAP after intra-renal albumin was much the same as after intra-renal saline, suggesting absence of systemic effects. In addition, final haematocrit was comparable (41±1% for both saline and albumin). Urine flow and GFR were unaffected by intra-renal infusion of LPC, saline or BSA. There was slight antinatriuresis during LPC, and enhanced natriuresis during recovery, but this was not different in the two groups. Urine flow of the right kidney was also unaffected by this protocol.

Discussion

The main hypothesis of the present study was that LPC is a more potent renal vasoconstrictor in hypoalbuminaemia than in normoalbuminaemia, because biologically active free LPC concentrations are increased. Indeed, when increasing concentrations of LPC were infused intra-renal, a stronger decrease in RBF was demonstrated in NAR than in Sprague-Dawley rats was due to albumin. Therefore, to study the effect of albumin on LPC-induced renal vasoconstriction, we infused LPC in the maximum dosage used in the first protocol, i.e. 10 mmol/l for 60 min in two groups of NAR, followed either by intra-renal 4% BSA or intra-renal saline. Changes induced at the end of the infusion period are listed in Table 2. Again, LPC caused a significant reduction in RBF, accompanied by a significant increase in RVR (P < 0.01, LPC vs baseline) (Table 2). Compared with the first protocol, where 10 mmol/l LPC was infused for only 20 min, the recovery of RBF was delayed [90 min (range: 70–90) after stopping LPC 95% recovery had been achieved in the first protocol as compared with only 42±8% in the second protocol in the same period; P < 0.05]. Intra-renal infusion of 4% BSA, completely restored RBF in for 75 min (30–90); at this time point spontaneous recovery was only 33±13% (P < 0.05; Figure 4). The renal venous and femoral arterial plasma concentration of BSA obtained at the end of the experiments were 6.3±0.6 and 2.5±0.8 g/l, respectively (n = 6). MAP after intra-renal albumin was much the same as after intra-renal saline, suggesting absence of systemic effects. In addition, final haematocrit was comparable (41±1% for both saline and albumin). Urine flow and GFR were unaffected by intra-renal infusion of LPC, saline or BSA. There was slight antinatriuresis during LPC, and enhanced natriuresis during recovery, but this was not different in the two groups. Urine flow of the right kidney was also unaffected by this protocol.

Table 2. Comparison of recovery from LPC (10 mmol/l) infusion in NAR with intra-renal infusion of either saline (Group 1; n = 9) or 4% BSA (Group 2; n = 8)

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<tr>
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<th>Group 1</th>
<th>Group 2</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>LPC</td>
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<tr>
<td>MAP (mmHg)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>110±2</td>
<td>117±4</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>48±1</td>
<td>45±2</td>
</tr>
<tr>
<td>RBF (ml/min/g kidney)</td>
<td>8.2±0.7</td>
<td>5.6±0.7a</td>
</tr>
<tr>
<td>RVR (U)</td>
<td>14.4±1.3</td>
<td>24.9±4.5a</td>
</tr>
<tr>
<td>Urine flow (ml/min)</td>
<td>10.2±1.1</td>
<td>7.9±2.2</td>
</tr>
<tr>
<td>GFR (ml/min/g kidney)</td>
<td>0.77±0.18</td>
<td>0.70±0.17</td>
</tr>
<tr>
<td>Natriuresis (μmol/min)</td>
<td>0.9±0.2</td>
<td>0.6±0.2</td>
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</table>

*a P < 0.05 vs baseline.

b P < 0.05 vs LPC.

P < 0.05 vs saline.
Sprague–Dawley. In addition, albumin was capable of restoring LPC-induced renal vasoconstriction.

Despite the well-documented effects of LPC on vasoconstriction and EDR [3,5], Handa et al., demonstrated that an i.v. bolus or continuous i.v. infusion of LPC only weakly increased sodium and water excretion without changing blood pressure and renal haemodynamics [10]. However, they dissolved LPC with BSA, which tightly bound LPC [4], thus preventing access of LPC to the vascular wall. Nevertheless, increased LPC in lipoproteins and cell membranes may well impair RBF. To our knowledge, no other studies have been performed to assess the effect of LPC on renal haemodynamics. We therefore investigated the effect of LPC on RBF in normal and analbuminaemic rats. Because of the absence of albumin in NAR, we assumed that less LPC would be bound in the circulation and that unbound LPC would ‘escape’ into the vessel wall, and hence induce vasoconstriction. Currently, we observed that intra-renal infusion of LPC dose-dependently decreased RBF and that this effect is specific for LPC as intra-renal infusion of LPE did not cause this effect. Previous studies in endothelial cells have documented that LPC has specific effects not shared by other lysophospholipids that are present in cell membranes such as LPE [11]. Infusion of LPC decreased RBF more profoundly in NAR than in Sprague–Dawley. Renal LPC content and RBF were similar under baseline conditions in NAR and Sprague–Dawley rats. Thus, when not challenged with exogenous LPC there is apparently enough LPC-binding capacity in the NAR to maintain normal renal vascular resistance. One sink for LPC in the NAR is the red cell membrane [8]; however, there may well be others. Apparently these putative sinks do not have much reserve capacity, because intra-renal LPC infusion in NAR resulted in a large increase in renal LPC content, whereas this was not the case in Sprague–Dawley rats. Because we analysed whole kidney homogenate it is unknown whether the exogenous LPC accumulated in the intra-renal vascular wall or also entered other compartments. In hyperlipidaemic nephrotic subjects LDL LPC content was increased from ~0.035 to 0.095 mmol/l [7]. Therefore, it was surprising that the infusion of LPC at an assumed maximum local concentration of 0.01 mmol/l (see Subjects and methods) could have such strong effects on RBF. Possibly accumulation over the infusion period produced much higher LPC concentrations locally. However, besides the amount infused, duration of the infusion also had a separate effect that can be appreciated when comparing the effect on diuresis and GFR in the first and fourth experiments.

After stopping the infusion of 10 mmol/l LPC (which was infused for 20 min), it took longer in NAR before RBF had recovered, than in Sprague–Dawley rats [90 (range: 70–90) vs 45 min (40–60)]. However, after infusing 10 mmol/l LPC for 60 min in NAR, recovery of RBF by saline was delayed and RBF did not return to baseline, even after 120 min. One way to explain the observations is that after longer infusion periods more LPC accumulates in the vascular wall.

We also studied the effect of albumin on renal vasoconstriction induced by a 60-min infusion of 10 mmol/l LPC. Recently, we have found that in the absence of albumin, LPC levels that occur in normal plasma completely block EDR in rat aortic rings. Albumin protected EDR against even higher LPC concentrations and salvaged EDR after exposure to LPC levels that occur in circulating LDL of subjects with nephrotic syndrome [5]. Previously, we found
restorative effects of albumin on whole blood hyperviscosity and reduced red cell deformation in NAR [8]. In the present study LPC-induced renal vasoconstriction was completely prevented by i.v. albumin. Although this could well be due to albumin binding of LPC because the plasma albumin level achieved was > 20 g/l, these experiments clearly induced volume expansion that may also have increased RBF. However, in NAR LPC-induced renal vasoconstriction was completely restored by intra-renal infusion of a low concentration of albumin (renal venous and femoral arterial plasma albumin concentration at the end of the experiments being 6.3 and 2.5 g/l, respectively) in contrast to saline. Such low levels of albumin are usually exceeded in subjects with the nephrotic syndrome [12]. This indicates that nephrotic subjects may actually have enough albumin to prevent LPC-induced renal vasoconstriction, even though the LPC levels of VLDL and LDL are increased in these patients [7]. Intra-renal albumin infusion restored RBF after ~75 min (range: 30–90), presumably by shifting LPC out of cell membranes. However, this restoration was less rapid than that seen in the normo-albuminemic Sprague–Dawley rats during intra-renal saline [a recovery time of ~45 min (40–60) after 10 mmol/l LPC for 20 min], presumably due to increased uptake of LPC in the membrane during the longer (60 min) infusion period. Thus, the data suggest that a low level of albumin may suffice to prevent LPC-induced vasoconstriction in vivo. It is unlikely that the recovery of RBF in the last set of experiments was due to differences in volume expansion, as changes in haematocrit were similar in the saline and albumin experiments.

Uptake of LPC into the endothelial membrane had been demonstrated by Kugiyama et al. [13]. They showed that LPC can shift from ox-LDL to the surface membrane of endothelial cells and that the transfer of LPC into the endothelial surface membrane is responsible for ox-LDL-mediated inhibition of NO release. There are several pathways through which LPC can impair EDR. LPC impaired the high affinity arginine transport of endothelial cells, disturbing arginine uptake [14]; and LPC blocked G-protein-dependent signal transduction pathways by selective uncoupling of the receptor to the G-protein [15]. LPC may also affect endothelial function by stimulating endothelial protein kinase C (PKC) activity [16], which caused sustained vasoconstriction upon stimulation of agonists such as phenylephrine, angiotensin and thrombin. PKC is also associated with increased superoxide production [17], which may interfere with NO bioavailability. Indeed, LPC can activate endothelial NAD(P)H oxidase, enhancing superoxide production [17]. However, in aortic rings superoxide production did not appear to be the specific cause of reduced EDR, even though it was dose-dependently increased by LPC [5]. Defining the mechanisms by which on the one hand LPC induces vasoconstriction and on the other inhibits vasodilatation may allow clearer delineation of functional and structural effects of oxidized lipoproteins. Traditionally, vasoconstriction in nephrosis and cirrhosis has been ascribed to a decrease in effective arterial blood volume. However, disturbed LPC handling in nephrosis [7] and cirrhosis [18] may provide an alternative pathway.

Low concentrations of LPC (0.1–10 μmol/l) have been shown to disturb EDR [3]. Previously, we have found that in NAR LPC content was increased in LDL from ~0.03 to ~0.10 mmol/l [6] and in erythrocytes from ~0.03 to 0.05 mmol/l blood [8]. Similar concentrations of LPC were found to disturb EDR, which was prevented by albumin at a concentration of only 6 g/l [5]. We also found that this low concentration of albumin could restore EDR in rings exposed previously to LPC (unpublished). In the present study we found that an intra-renal infusion of 10 mmol/l LPC strongly reduced RBF in the NAR and that an intra-renal albumin concentration of ~6 g/l was capable of restoring RBF. The recovery observed with saline, however, indicates that even in the absence of albumin LPC is not irreversibly incorporated into cell membranes. A decrease in LPC content in the membrane will lead to restoration of the membrane function, e.g. restoration of G-protein-dependent signal transduction, or less superoxide production by NAD(P)H oxidase. Albumin strongly binds LPC, by a molar ratio of 1:4 (albumin/LPC) [4]. Presumably that is why intra-renal albumin infusion accelerated recovery of RBF.

Exogenous LPC decreased in vivo kidney perfusion by a maximum of 25% in control (Sprague–Dawley) rats. This effect is not restricted to the rat kidney. In isolated perfused rat heart, low concentrations of LPC (3–50 μmol/l) reduced heart rate, coronary flow and contractile function [19]. In isolated perfused posterior cerebral arteries LPC (10 μmol/l) enhances myogenic tone through the release of endothelin-1 [20]. The present data indicate that, under pathophysiological circumstances, endogenous LPC could contribute to the development of systemic vasoconstriction and cardiac ischaemia. Whether increased LPC, considering the important role of LPC in the biological actions of ox-LDL, contributes to the haemodynamic profiles in hypercholesterolaemia remains to be specifically investigated.

In conclusion, under baseline conditions RBF in NAR is not disturbed and renal LPC content is normal. However, when the kidney is exposed to levels of LPC similar to those found in nephrotic subjects much more pronounced renal vasoconstriction and accumulation of LPC occurs than in control rats. Addition of albumin prevents and restores LPC-induced renal vasoconstriction, suggesting that albumin is very potent in buffering LPC.

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