High-affinity interaction of long-chain fatty acids with serum albumin in nephrotic syndrome

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1. We have examined the effect of hypoalbuminaemia, a hallmark of nephrotic syndrome, on the albumin-fatty acid equilibrium in the plasma of 11 adult patients with nephrosis compared with 12 healthy subjects and six subjects with normoalbuminaemic hyperlipoproteinaemia.

2. We used a dialysis exchange rate method which allows the evaluation in relative terms of the binding affinity of albumin for plasma fatty acid and the fatty acid availability, tentatively equated with the unbound fatty acid fraction.

3. In nephrotic patients, an increase (P<0.001) in albumin affinity for fatty acid was seen compared with healthy subjects, which was negatively correlated with albuminemia (r=−0.69, P<0.02). No change in fatty acid availability was seen for the group as a whole, but individual values showed a wide scatter, with the highest values in four patients with the highest fatty acid-albumin molar ratios. The increase in albumin affinity for fatty acid was specific to nephrotic syndrome since no such effect was seen in subjects with hyperlipoproteinaemia, who only showed a moderate increase (P<0.01) in fatty acid availability.

4. The increased albumin affinity for fatty acid in nephrotic syndrome supports the hypothesis that an albumin component with lower affinity for fatty acid might filter out through the diseased glomerular membrane and leave the high-affinity albumin in plasma.

INTRODUCTION

Hypoalbuminaemia is a major cause of metabolic disorders in nephrotic syndrome (NS), primarily through associated hyperlipoproteinaemia, atherosclerosis and renal dysfunction [1]. Reports on the influence of hypoalbuminaemia on fatty acid (FA) transport in plasma are scarce. Most long-chain FAs, which are major energy-yielding substrates, are largely water-insoluble at neutral pH and circulate in blood essentially in association with serum albumin [2]. An unbound FA (UFA) fraction, presumably in water-soluble monomeric form, coexists in dynamic equilibrium with the albumin-bound fraction [3]. Efforts to quantify UFAs, particularly under conditions in vivo, have been hampered by their levels which, on average, are probably lower than 10⁻⁹ mol/l [4, 5]. Because of FA insolubility, one cannot characterize the albumin/FA binding equilibrium by measuring free and bound ligand concentrations. Alternatively, one can evaluate the binding equilibrium in relative terms, using the dialysis exchange rate technique described by Brodersen et al. [6–8]. In this study, we have used the dialysis method to examine whether hypoalbuminaemia could modify the albumin/FA equilibrium in the plasma of adult nephrotic patients compared with healthy control subjects and subjects with normoalbuminaemic hyperlipoproteinaemia.

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MATERIALS AND METHODS

Materials

Globulin-free human serum albumin (batch 93H9345), containing less than 0.005 mol FA/mol albumin, was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. [1-¹⁴C]Palmitic acid (> 98.7% radiochemically pure (60 mCi/mmol)] was from New England Nuclear Research Products (Du Pont de Nemours, Dreieich, Germany). Unlabelled palmitic acid (chemically >99% pure) was from Fluka Chemika (Buchs, Switzerland). Microdialysis apparatus were locally toileed from Plexiglas according to the method of R. Brodersen (personal communication). Dialysis chambers had a volume of 570 μl on each side of a cellulose triacetate membrane (cut-off 20 kDa; type 14549 from...
Acetylcellulose membranes give measurable dialysis rates in a range of standard albumin concentrations from 10 to 600 μmol/l, with total recovery of radioactivity from left and right compartments ranging from 70 to 90%. The use of acetylcellulose membranes has been documented previously.

Patients

We studied 11 patients with NS (seven women and five men, age range 15–70 years; group NS). Using the following current diagnostic criteria, the underlying renal disease was classified as primary NS: (i) proteinuria > 3.3 g/24 h, albuminaemia < 435 μmol/l with no or mild renal failure (creatininaemia < 200 μmol/l) and (ii) renal histological lesion (membranous nephropathy, 4; SLE minimal change disease, 4; amyloidosis, 2). None had diabetes mellitus, liver disease, was taking corticosteroids, oral contraceptive or lipid-lowering agents. Two control groups included 12 healthy subjects (five women and seven men, age range 21–89 years; group C1) and six normoalbuminaemic (> 540 μmol/l) hyperlipoproteinaemic subjects (two women and four men, age range 32–48 years; group C2). Blood (citrated) samples were drawn at 09.00 hours after an overnight fast while subjects had been resting for at least 20 min. The study protocol was approved by the Ethics Committee of our institution, and written informed consent was obtained from all subjects.

Microdialysis method

Methodological principle. The albumin/FA binding equilibrium can be assessed quantitatively in relative terms using the reserve albumin concentration (p), defined as the concentration of a reference albumin preparation which, in a buffered solution, binds a trace amount of FA (e.g. [14C]palmitate), as tightly as it is bound to albumin in a plasma sample [10]. p is determined by measuring the rate of dialytic exchange between two identical plasma samples after addition of the labelled tracer on one side of the membrane. The dialysis exchange rate for a given plasma, which depends upon the free ligand concentration, is compared with rates measured with varying concentrations of standard albumin. The p value is the albumin concentration which gives the same rate as that measured with the plasma sample. From p, palmitate availability (C/p) can be deduced, where C is the concentration of bound (total) palmitate. In practice, the p value for one given FA (e.g. palmitate) has been extended to p*, the mean value for the major long-chain FAs circulating in human plasma [7]. This is acceptable, since pilot studies have shown that the variation of p did not exceed 10% for saturated and monounsaturated FAs with 16- or 18-carbon chain lengths [6]. Consequently, we can let C* represent the total FA concentration in a plasma sample and measure p (= p*) for palmitate. It follows that C*/p* is the total FA availability in the plasma sample, which can be tentatively equated with the UFA fraction for analysis of FA transport [7]. The relative binding affinity, L*, of albumin for plasma FA can be calculated using the formula: $L^* = (p*/P) - a(C*/P)$, where P is the albumin concentration in plasma. The numerical value of C* is −0.05, as published [7] and verified in this study.

Dialytic exchange rate determination. Dialysis experiments were carried out as follows. A stock solution of [14C]palmitate–albumin was prepared by evaporating to dryness in a stream of nitrogen a trace amount of [14C]palmitic acid (25 μCi) in 0.25 ml of ethanol. To the dried extract was added 0.5 ml of a 50 μmol/l albumin solution in 3.3 mmol/l sodium phosphate at pH 7.4. Portions (40 μl) of the [14C]palmitate–albumin solution were stored at −20 °C. Determination of p* was carried out in triplicate in plasma samples diluted 1:1 with 100 mmol/l sodium chloride and 66 mmol/l sodium phosphate (pH 7.4). To 700 μl of the diluted sample was added 11 μl of the [14C]palmitate–albumin solution, and the mixture was kept at room temperature for 10 min to allow binding equilibrium of labelled and non-labelled palmitate to albumin. A 200-μl aliquot of this mixture was placed in the left compartment of each of three dialysis cells, and these were rotated for 10 min at 120 rpm in a water bath at 37°C in order to preheat the samples. An identical plasma sample without [14C]palmitate was preheated simultaneously. The dialysis was started by adding 200 μl of the latter sample to each of the right compartments. Dialysis was allowed to proceed for 1 h under rotation of the cells (120 rpm). After this time, 100-μl aliquots of the fluid were drawn from each compartment, mixed with 3 ml of scintillation fluid and counted. The ratio, r, of the left/right-side counts was calculated.

Determination of the dialytic exchange rate was carried out simultaneously with increasing concentrations of standard albumin in 66 mmol/l of sodium phosphate buffer (pH 7.4), identical on both sides of the membrane. A constant amount of [14C]palmitate was added to the left-side solutions, as described above, together with an equal amount of unlabelled palmitate to the right-side solutions. Exchange rates were plotted against albumin concentrations, supplying the linear reference curve for the determination of p* (Fig. 1). Triplicate determinations of p* agreed to within 3.9% (n = 29), and its mean value for the C1 group (347 ± 15 μmol/l) was in good agreement with that reported for 33 healthy women (351 ± 50 μmol/l) [6].

Other procedures. Serum albumin was measured by immunoturbidimetry (protocol and reagents from Behring, Marburg, Germany). Serum concentrations of cholesterol, triacylglycerol and FA were measured

Sartorius, Goettingen, Germany).
Table 1. Characteristics of subjects studied. Values are given as means ± SEM. Statistical significance: *P < 0.05 and **P < 0.001 compared with CI.

<table>
<thead>
<tr>
<th></th>
<th>Control subjects (CI) (n = 12)</th>
<th>Hyperlipoproteinaemic subjects (C2) (n = 4)</th>
<th>Nephrotic patients (NS) (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria (g/day)</td>
<td>&lt;0.03</td>
<td>0.03</td>
<td>6.3 ± 0.4</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>4.6 ± 0.3</td>
<td>7.5 ± 0.6*</td>
<td>11.1 ± 1.2**</td>
</tr>
<tr>
<td>Serum triacylglycerols (mmol/l)</td>
<td>0.73 ± 0.07</td>
<td>3.1 ± 0.8*</td>
<td>3.6 ± 0.27**</td>
</tr>
</tbody>
</table>

Fig. 1. Standard curve for determination of $\rho^*$, the reserve albumin for binding of long-chain FAs in appropriately diluted plasma samples. The ratio of left/right compartment radioactivities after non-equilibrium dialysis is plotted against the concentration of standard albumin, equal on both sides of the membrane.

Fig. 2. Individual plasma concentrations of albumin (○) and total FAs (○) in healthy control subjects (CI), hyperlipoproteinaemic subjects (C2) and patients with NS. The large circles (and bars) represent the means (± SEM).

Fig. 3. Individual values (arbitrary units) of (a) albumin affinity for fatty acids ($L^*$) and (b) FA availability ($C^*/\rho^*$) in plasma from healthy control subjects (CI), hyperlipoproteinaemic subjects (C2) and patients with NS. The large circles (and bars) represent the means (± SEM). Statistical significance is plotted.

RESULTS

Characteristics of the study groups are presented in Table 1. Major increases in serum lipids were measured in the NS group and, to a lesser extent, in the C2 group. Figure 2 shows the distribution of albumin and FA concentrations in plasma. As expected, albumin is within the normal range in the C1 and C2 groups, and is approximately one-third of normal in the NS group. Compared with values in the C1 group, FAs are lower ($P < 0.05$) in the NS group and higher ($P < 0.05$) in the C2 group, resulting in FA/albumin molar ratios of 0.99 and 0.74 in the NS and C2 groups, respectively, compared with 0.45 in the C1 group. Fig. 3(a) shows that the
albumin affinity for FA, $L^*$, is elevated very significantly ($P<0.001$) in the NS group ($0.79 \pm 0.05$, arbitrary units) compared with values in both the C1 ($0.51 \pm 0.02$) and C2 ($0.47 \pm 0.05$) groups. $L^*$ values in the NS group were negatively correlated with albuminemia ($r = -0.69$, $P<0.02$). Fig. 3(b) shows that, in the NS group, the mean value of 1.30 ± 0.21 for FA availability, $C^*/p^*$, is not significantly different from that in the C1 group ($0.91 \pm 0.12$). The highest individual $C^*/p^*$ values were found in four nephrotic patients who also showed the highest $C/p$ ratios for this group, with a mean value of 1.48 ± 0.41 compared with 0.68 ± 0.29 for the remaining seven subjects. In contrast, the value of 1.79 ± 0.30 for $C^*/p^*$ in the C2 group was significantly increased ($P<0.01$) compared with C1.

**DISCUSSION**

In model experiments, UFA concentration seems to increase with the FA/albumin ratio [5]. The present results indicate that this relationship may hold true for hyperlipoproteinaemic subjects in whom increased FA availability is clearly associated with an increased FA/albumin ratio, without a change in the albumin affinity for FA. Our finding of a higher affinity of albumin for binding FA in nephrotic patients suggests a more complex situation. Theoretically, if FA availability depends inversely upon FA binding to albumin, an increased albumin affinity for FA should result, all else being equal, in low FA availability. In cases of hypoalbuminaemia, a high-affinity binding could therefore contribute to maintaining a normal FA availability, as was observed in seven nephrotic patients with FA availability in the control range (Fig. 3). Alternatively, a high FA availability may be found, together with a high albumin/FA affinity, if hypoalbuminaemia co-exists with an unusually high FA/albumin ratio, as was observed in the other four nephrotic patients with higher FA availability.

The reasons for the increased albumin affinity for FA in NS are at present unclear. Human albumin, a non-glycosylated, very soluble protein is encoded by a single gene, the two alleles of which may be polymorphic, leading to the expression of albumin variants [12]. Chemically, these variants are allelomorphs of albumin and may be synthesized as major constituents under genetic control. Genetic variants co-exist in plasma with more transient isomeric forms which can be detected using various physicochemical approaches [13]. For example an ‘F’ (for fast) form moves rapidly upon gel electrophoresis, and also differs from the ‘N’ (for normal) form in being precipitated readily by 3 mol/l KCl. Conceivably, such a protein heterogeneity may have functional consequences. Tests based on the transport function, and in particular analysis of binding equilibria for various FAs to human albumin [14], suggest a model with two albumin components, one with high affinity and one with low affinity, with approximately 0.65 of the albumin having high-binding affinity. The high-affinity albumin component binds 1 mol of decanoate, 1 mol of octanoate or 2 mol of hexanoate more than the low-affinity component, and C1− competes with the high-affinity binding for all three ligands. It is tempting to speculate that, in NS, the albumin component with the lower affinity for FA might filter out through the diseased glomerular membrane and leave the high-affinity albumin in the plasma. This supports the hypothesis of Ghiggeri et al. [15] that, in the plasma of nine nephrotic children affected with minimal change nephropathy, a less anionic serum albumin isoform, prevented from binding FA, could be the main component of urinary albumin. In these children, the high number of FAs per mol of albumin (mean 8.6 compared with 2.2 in normal age-matched children) also suggests that a high-affinity albumin circulates in plasma.

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**REFERENCES**