Plasma factors controlling atrial natriuretic peptide (ANP) aggregation: role of lipoproteins

Emanuela Maioli a, Claudia Torricelli a, Annalisa Santucci b, Paola Martelli b, Adriana Pacini a,*

a Institute of General Physiology, University of Siena, via Aldo Moro, 53100 Siena, Italy
b Department of Molecular Biology, Section of Biological Chemistry, University of Siena, via Fiorentina 1, 53100 Siena, Italy

Received 11 October 2000; received in revised form 23 February 2001; accepted 1 March 2001

Abstract

We have previously shown that human plasma atrial α-natriuretic peptide (α-hANP) sequestering is a protective phenomenon against amyloid aggregation. In the present work, the possible role of lipoproteins as α-hANP binding factors has been investigated in vitro using an experimental model, developed in our laboratory, that allows to work at physiological concentrations. This approach consists of gel filtration on Sephacryl S-300 HR of big α-[125I]hANP generated in phosphate buffered saline or in human normal plasma supplemented or not with lipoproteins. The results of these experiments indicate that high density lipoproteins (HDL) are responsible for the ANP binding phenomenon observed in vitro, while low density lipoproteins and very low density lipoproteins do not directly interact with ANP. Moreover, the HDL remodeling process occurring in vitro has been analyzed during plasma incubation by monitoring the redistribution of lipids and apolipoproteins among the HDL subclasses. The changes in HDL size and composition observed in incubated plasma were compared with the redistribution of endogenous and labeled big ANP. The obtained results revealed that both tend to follow the molecular rearrangement in plasma of apolipoprotein A-I containing particles and suggested that, among HDL species, the small particles are mainly involved in the ANP binding phenomenon. This hypothesis was further demonstrated by ligand blotting experiments that confirmed the existence of differences in the ability of HDL particles to bind α-[125I]hANP. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Amyloidosis; Atrial natriuretic peptide; High density lipoprotein; Apoprotein

1. Introduction

Isolated atrial amyloid (IAA) is a relatively common disease in the aging human heart [1–3] and atrial α-natriuretic peptide (α-ANP) is the major immunoreactive subunit of amyloid fibrils [4–6]. Analogously to other amyloid endocrine forms, it is unclear why some peptide hormones give rise to amyloid fibrils. It is known that peptide aggregation and fibril formation are greatly influenced by peptide concentration, pH, oxidation, ionic strength and temperature, as well as by the presence of amino acid substitutions [7–13]. Indeed, a relationship between certain cardiac disorders, associated to high ANP plasma levels, and the incidence and the degree of IAA has been described [1,3].

Although it is not known whether ANP aggregates in biological fluids, the circulating peptide exists as
either a monomeric or macromolecular form at picogram physiological concentration [14,15]. In a previous work [16] we investigated the nature of high molecular weight (big) forms of ANP by setting up an in vitro model suitable for studying the aggregation process of radiolabeled ANP at physiological concentrations. We showed that the process is analogous to that occurring for other amyloidogenic peptides [17-21]. In the presence of a preformed nucleus (seed), the aggregation is suddenly initiated, following a first order kinetic model, while in the absence of seed, a lag time, longer than 8 days, is observed at picogram ANP levels [16]. Such an in vitro study also suggested that a part of the big ANP is the expression of binding to some plasma components and that this binding competes with the ANP aggregation process. Moreover, we confirmed previously reported results [22], i.e., that the plasma binding factors are not specific carriers for ANP since the binding is not saturable or reversible, thus acquiring the peculiarity of a sequestration phenomenon. Hence, these ANP binding factors may have an important role in impairing amyloid formation and growth. In fact it is probable that only the ANP not bound to proteins can take part in the polymerization process generating amyloid fibrils, as hypothesized for the amyloid β-peptide (Aβ) responsible for amyloidosis associated to Alzheimer’s disease [23,24].

Most of the information on sequestering factors comes from studies on Aβ. Several circulatory proteins, such as apolipoprotein (apo) E, apo J, apo A-I, apo A-II and gelsolin, have been shown to bind and form complexes with Aβ [24-30]. A recent study suggests that the interaction of transthyretin (TTR) with high density lipoproteins (HDL) occurs via association with apo A-I [31]. To date, no information exists about the interaction of such endogenous factors and ANP. However, our previous findings [16] demonstrate that human albumin did not bind monomeric labeled ANP. These data are in line with some recent reports [26,28], which demonstrate the inability of albumin to bind monomeric Aβ. On the other hand, the ubiquitous presence of some of the apolipoproteins in chemically diverse cerebral and systemic amyloid deposits [32-36], together with the demonstrated binding to soluble Aβ, give impetus to the idea that these compounds may be designated to sequester several amyloidogenic peptides in vivo.

On the basis of these observations, in the present work we applied our previously presented experimental model [16] to test the effect of HDL, low density lipoproteins (LDL) and very low density lipoproteins (VLDL) on the chromatographic distribution of exogenous labeled ANP when incubated in buffer and in human plasma at physiological concentration (picograms). To further investigate the relationship between lipoprotein and α-[125I]hANP, ligand blotting experiments were carried out.

2. Materials and methods

2.1. Materials

All chemicals used were reagent grade and ultrapure water (Milli Q System Millipore, USA) was always used. α-[125I]hANP (specific activity 2000 Ci/mmol) and the α-hANP radioimmunoassay (RIA) kit were obtained from Eiken Chemical (Japan). Dextran (molecular mass 65-85 kDa), activated charcoal (100-400 mesh), bovine serum albumin (BSA) and lipoproteins isolated from human plasma (HDL, LDL, VLDL) were obtained from Sigma (Italy). Gel filtration calibration kits and the HiPrep Sephaeryl S-300 High Resolution (HR) gel filtration column, Kodak X-Omat AR film, intensifying screens and hypercassette autoradiography were supplied by Amersham Pharmacia Biotech (Italy). Immunodiffusion plates and standards for apo A-I and apo A-II assay were obtained from Daiichi Pure Chemicals (Japan). Enzymatic kits for total cholesterol and triglycerides were from Menarini Diagnostics (Italy). The phospholipid assay kit was from Diacron (Italy). Visking dialysis tubing (MWCO 12 kDa) were supplied by Tecnochimica Moderna (Italy). A human pooled plasma sample was obtained from 20 healthy volunteers with their informed consent. EDTA and aprotinin (500 kIU/ml of blood) were used. The same pooled plasma, stored at −70°C in multiple aliquots, was used for all subsequent analyses.

2.2. ligand binding of α-[125I]hANP to lipoprotein in solution

Big ANP was obtained by incubating 100 µl of
plasma or phosphate buffered saline (PBS; 0.15 M NaCl, 0.05 M Na-phosphate and NaN₃ (0.02%, w/v), pH 7.0) with 100 μl of α-[¹²⁵I]hANP (10 000–50 000 cpm) at 37°C in a water bath for 24 h, as previously reported [16]. In parallel experiments, physiological amounts of HDL (230 μg), LDL (230 μg) and VLDL (60 μg) were added to 100 μl of plasma or PBS. The monomeric and big α-[¹²⁵I]hANP were separated by adding a dextran-coated charcoal (DCC) suspension (α-[¹²⁵I]hANP/DCC, 1:10 v/v). The charcoal-adsorbed α-[¹²⁵I]hANP was pelleted by centrifugation at 1000 × g for 15 min at 4°C and the supernatant containing big α-[¹²⁵I]hANP was counted in a γ-counter. This separation method and its efficiency have been reported previously [16,22]. The big α-[¹²⁵I]hANP amount was expressed as percent of the total radioactivity added to the incubation mixtures.

In a separated set of experiments big α-[¹²⁵I]hANP generated in PBS or PBS supplemented with HDL was incubated at 37°C for 24 h in plasma.

2.3. Gel chromatography

Samples containing big α-[¹²⁵I]hANP were applied to a 1.6 × 60 cm column of Sephacryl S-300 HR (fractionation range, 10–1500 kDa). The column was equilibrated at room temperature in PBS, pH 7.0. The same buffer was used for the elution step. The flow rate was 30 ml/h; loading volumes were 1 ml. Fractions (1 ml) were collected, directly counted for ¹²⁵I and the radioactive elution profile drawn. The recovery of radioactivity ranged from 80 to 90% of the total applied. The apparent molecular size of the labeled material in the fractions was determined by comparing its elution volume with that of commercially available gel filtration protein calibration mixtures (protein size range, 25–669 kDa). For this purpose, a plot of log molecular mass vs. fraction number was constructed and the points fitted using curvilinear least-squares regression analysis. Big α-[¹²⁵I]hANP, eluting in column fractions 34–40, 41–50, 51–60 and 61–75, included material >1500 kDa, 460–1100 kDa, 170–460 kDa and 40–170 kDa in molecular size, respectively.

In order to accumulate sufficient amounts of endogenous big ANP, 12 ml of pooled plasma either native or incubated without α-[¹²⁵I]hANP at 37°C for 24 h were consecutively chromatographed on Sephacryl S-300 HR column in 1 ml aliquots. Corresponding fractions were pooled and stored at −20°C. According to the molecular mass ranges reported above, fractions 34–40 (pool 1), 41–50 (pool 2), 51–60 (pool 3) and 61–75 (pool 4) either from native or incubated plasma were accumulated. Finally these eight samples were dialyzed against ultrapure water, lyophilized, and assayed for ANP, apolipoprotein and lipid content.

2.4. Immunoreactive ANP (ir-ANP) analysis

The endogenous big ANP was measured by a commercially available α-hANP RIA kit as previously reported [22]. The values were reported as total amount in each of the eight lyophilized samples.

2.5. Apolipoprotein and lipid analyses

Apo A-I and apo A-II were measured by radial immunodiffusion using commercially available kits. Total cholesterol (T Chol), triglycerides (TG) and phospholipids (PL) were measured by enzymatic colorimetry using commercially available Trinder-class assays. For all kits we followed the manufacturer’s instructions. The protocols were partially modified to adapt suitable dilutions to our samples. All values were reported as total amount in each of the eight lyophilized samples.

2.6. Gel chromatography of commercial HDL

In order to characterize the commercial preparation of HDL, 1 ml of the solution (protein concentration: 10 mg/ml) was applied to a Sephacryl S-300 HR column under identical conditions as described in Section 2.3. The column was equilibrated and eluted with PBS supplemented with 1 mM EDTA (pH 7.0). Fractions of 1 ml were collected and monitored for protein content by measurement of absorbance at 280 nm. The chromatographic profile showed that an initial small peak was eluted at the void volume and was followed by a broader, larger-sized peak. This latter finding suggested a coalescing of more peaks in one (Fig. 4A). In agreement with the low lipid content, small HDL particles are detected only in trace amounts in HDL fractions prepared by density ultracentrifugation. Thus, the com-
mercial preparation, in addition to medium HDL, exhibited a modest degree of contamination by very large and small HDL particles. On the basis of this, fractions within the size ranges of >1100 kDa (fractions 31–40, pool a), 170–1100 kDa (fractions 41–60, pool b), 56–170 kDa (fractions 61–72, pool c) and 26–56 kDa (fractions 73–80, pool d) were assembled.

2.7. Ligand blotting experiments

After concentration, the pools were immobilized in nitrocellulose under vacuum. Membranes were saturated with 5% skim milk in PBS for 1 h at room temperature. After washing in PBS-0.05% Tween 20 (PBS-T), they were incubated for 5 h at 37°C with 500 000 cpm α-[125I]hANP. Then the membranes were extensively washed in PBS-T, dried and exposed overnight at –70°C to Kodak X-Omat AR autoradiography film and a single intensifying screen.

3. Results

3.1. Characterization of big α-[125I]hANP

When big α-[125I]hANP, prepared in plasma, was chromatographed on a Sephacryl S-300 HR column, we invariably identified at least three peaks of labeled material (Fig. 1A), in agreement with our previous results [16]. A minor peak in the region of medium HDL was unsteadily detected. The first peak (peak I), representing the largest α-[125I]hANP containing species, eluted at the void volume in fractions 34–40 (same position as dextran blue 2000); the second peak (peak II), which eluted in fractions 41–50, included big α-[125I]hANP of 460–1100 kDa and the third peak (peak III), eluting in fractions 61–75, included species 40–170 kDa in molecular size.

3.2. Incubation of α-[125I]hANP in plasma enriched with lipoproteins

Mixtures of monomeric α-[125I]hANP and plasma enriched with human HDL or VLDL or LDL were incubated at 37°C for 24 h. The Sephacryl S-300 HR elution profiles of big α-[125I]hANP are shown in Fig. 1. The presence of an excess of HDL in the incubation mixture resulted in an evident decrease

Fig. 1. Representative gel filtration profiles on Sephacryl S-300 HR of big α-[125I]hANP generated upon incubation at 37°C for 24 h in plasma (A), in plasma supplemented with LDL (B), with HDL (C), with VLDL (D), as described in detail in Sections 2.2 and 2.3. Big α-[125I]hANP elutes in three peaks: peak I (fractions 34–40, >1500 kDa), peak II (fractions 41–50, 460–1100 kDa) and peak III (fractions 61–75, 40–170 kDa). Similar profiles were obtained in three similar experiments.
of peaks I and II and a significant increase of peak III (Fig. 1C). When the experiment was repeated using VLDL essentially similar results were obtained (Fig. 1D). Nevertheless a lower increase in big $\alpha$-[$^{125}$I]hANP eluting in peak III was observed. Incubation for 24 h in the presence of LDL had no appreciable effect on the big $\alpha$-[$^{125}$I]hANP chromatographic profile (Fig. 1B).

3.3. Incubation of $\alpha$-[$^{125}$I]hANP in PBS in the presence of lipoproteins

Mixtures of monomeric $\alpha$-[$^{125}$I]hANP and PBS were incubated for 24 h at 37°C with a physiological concentration of human HDL or VLDL. The big $\alpha$-[$^{125}$I]hANP formed was chromatographed on Sephacryl S-300 HR. On the basis of the results obtained when the incubation was carried out in plasma enriched with LDL (Fig. 1B), LDL were not tested. Radioactive profiles are shown in Fig. 2. In the control incubations, carried out in the absence of lipoproteins (Fig. 2A), peak I was the only labeled component eluting in the area of the $V_0$. No modification of the big $\alpha$-[$^{125}$I]hANP profile was observed in the presence of VLDL (data not shown). On the contrary, in the presence of HDL the elution pattern (Fig. 2B) showed a shoulder in the descending limb of peak I, corresponding to plasma peak II (Fig. 1A). This shoulder was accompanied by a decrease in peak I radioactivity. Thus, in PBS plus HDL the plasma radioactive profile was only partially restored, since no radioactivity was recovered in the region of peak III. Moreover, any attempt to generate peak III in buffer enriched with HDL, such as by prolonged incubation for up to 5 days, was unsuccessful (data not shown). Thus, it seems that peak III material could be generated only in the presence of plasma. Interestingly, when big ANP generated in HDL-enriched PBS was incubated in plasma, peaks I and II partially converted into peak III material (Fig. 2C). On the contrary, incubation in plasma of big ANP formed in PBS alone failed to generate peak III (data not shown).

3.4. Chromatographic distribution of apolipoproteins and lipids in native and incubated plasma

With regard to the nomenclature of plasma HDL subclasses used, we will refer to a recent study [37] which summarizes the knowledge about the chemical composition and size of HDL species.

As shown in Fig. 3, the incubation of plasma caused a change in the distribution of both apolipoproteins and lipids among the pools. Pool 1 (fractions 34-40) did not contain detectable amounts of apo A-I and apo A-II and likely consists of VLDL.
Fig. 3. Distribution of apolipoproteins, lipids and ir-ANP in native plasma (left) and after 24 h incubation at 37°C (right). Pools were prepared as described in Section 2.3. All values are reported as the total amount in each pool.
and LDL particles. This pool was enriched in lipids following incubation. Apo A-I immunoreactivity began to appear in pool 2 (fractions 41–50). In this pool, a slight increase in apo A-I, apo A-II and lipids was observed following incubation. In this region, very large HDL particles (large HDL) relatively poor in apo A-I, apo A-II and lipids are supposed to elute. Pool 3 (fractions 51–60) contained both apolipoproteins A-I and A-II and lipids. Upon incubation a decrease in both apolipoproteins and lipids is observed in this region. Pool 4 (fractions 61–75), containing the largest amount of apo A-I, was further enriched in apo A-I and apo A-II contents upon incubation, although apo A-II appeared to increase at a smaller extent. The PL/apo A-I, T Chol/apo A-I, TG/apo A-I and apo A-II/apo A-I molar ratios were determined in order to identify the HDL subclasses. The values reported in Fig. 3 indicate that pool 3 (fractions 51–60) likely contains typical spherical K-migrating HDL particles (medium HDL), while small pre-β-migrating HDL (small HDL) probably elute in fractions 61–75 (pool 4). Indeed small HDL are poorer in both apo A-II and lipid with respect to the medium HDL. In particular, the PL/apo A-I molar ratio in the small HDL is significantly lower than that in the medium HDL (Table 1). Moreover, the first ratio may be overestimated because plasma albumin, also eluting in the region of small particles, is known to bind large quantities of phospholipids.

In conclusion, the main result of plasma incubation was a marked decrease in medium HDL and a striking increase in small HDL. A slight generation of further large HDL was also observed.

3.5. Chromatographic distribution of ir-ANP in native and incubated plasma

The ir-ANP was found in pools 2, 3 and 4 only, both in native and incubated plasma (Fig. 3). No ANP was detectable in pool 1, where apo A-I and A-II were also absent. The major immunoreactivity was present in native pool 3, in correspondence with medium HDL. This region decreases in ir-ANP upon incubation, apparently following the changes in distribution of apo A-I. A further relationship between apo A-I and ANP distribution was found in the region of small HDL. In particular, this region was enriched in both apo A-I and ir-ANP upon incubation.

3.6. Ligand binding of α-[125I]hANP to immobilized HDL

Ligand binding studies were performed in the samples prepared following gel filtration of commercial HDL as described in Section 2.6. As showed in Fig. 4B, binding of α-[125I]hANP was observed mainly to the small particle-rich pool and in decreasing order to larger HDL species, and finally no binding was seen to the particles eluting at the void volume. As can be seen by comparison of Fig. 4A,B, the signal intensity was not related to the protein content. These findings indicate that the HDL subspecies exhibit different binding affinity for α-[125I]hANP, in agreement with that already observed for plasma HDL. In fact, also under these conditions, the immobilized small HDL particles bound α-[125I]hANP with highest affinity.

Table 1
Relationship between composition and molecular size of HDL particles

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>51–60</td>
<td>460–170</td>
<td>N 0.33</td>
<td>56</td>
<td>71.1</td>
<td>15.7</td>
<td>medium</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td></td>
<td>0.50</td>
<td>56</td>
<td>58</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>61–75</td>
<td>170–40</td>
<td>N 0.15</td>
<td>27.5</td>
<td>8.9</td>
<td>2.9</td>
<td>small</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td></td>
<td>0.13</td>
<td>23.2</td>
<td>7.6</td>
<td>4.2</td>
<td></td>
</tr>
</tbody>
</table>

*See Section 2.3.

*a Ratios were calculated using the values reported in Fig. 3 for native (N) and incubated (I) pooled plasma.
4. Discussion

We have reported that different big forms of ANP circulate in the plasma [16]. In fact, upon gel filtration of human plasma on Sephacryl S-100 HR we obtained, in addition to a major immunoreactive component eluting in the void volume of the column, a second peak representing material of approx. 67 kDa in molecular size. These same forms were generated by in vitro incubation of labeled ANP in plasma. Since only material eluting in the void volume and no other form of big labeled ANP was obtained in buffer, we interpreted the former as aggregated ANP and the latter as ANP bound to plasma components. Fractionation experiments on Sephacryl S-300 HR showed that the bulk of labeled big ANP was again excluded from this more open gel (>1500 kDa). We confirmed, by seeding experiments with radiolabeled monomeric peptide, that this big form consists of ANP aggregates of amyloid or pre-amyloid nature since they generate through a nucleation dependent mechanism. Moreover, gel filtration on Sephacryl S-300 HR revealed that bound $\alpha$-[125I]hANP comprises several components of different molecular size, ranging from 40 to 1100 kDa. This result indicates that part of this bound material eluted from the Sephacryl S-100 HR column together with the aggregated forms. In the present paper, by using a Sephacryl S-300 HR column, we demonstrate that the chromatographic distribution of endogenous ANP corresponds to the profile of big $\alpha$-[125I]hANP generated in plasma, restrictedly to the bound ANP forms. In fact, no immunoreactivity is recovered in the void volume where, on the contrary, the labeled aggregated ANP is recovered. The lack of circulating ANP aggregates agrees with the already demonstrated inability of endogenous big ANP to act as a seed for polymerization [16]. Thus we can state, thanks to the present results, that endogenous big ANP consists only of bound ANP.

We previously pointed [16] to a protecting role of plasma binding factors against ANP fibrillar polymerization that leads in vivo to IAA [1–3]. The novel finding of the absence of circulating aggregates corroborates this hypothesis and indicates that amyloid preventing mechanisms are fully effective at plasma ANP concentrations. Although no direct information about the nature of ANP binding factors could have been drawn from that study [16], our preliminary unpublished findings suggested a lipoprotein involvement in human ANP biology.

In the present work we have tested whether lipoproteins were actually involved in the ANP binding phenomenon. While no association between LDL and $\alpha$-[125I]hANP was found, the enrichment of plasma with HDL or VLDL caused an increase of bound ANP, suggesting that ANP associates with components of HDL and VLDL. However, control experiments in PBS showed no association of $\alpha$-[125I]hANP with VLDL. On the contrary, HDL addition to PBS gave rise to iodinated ANP species eluting close to the void volume only, likely as a consequence of HDL aggregation in saline medium at 37°C. In fact, the chromatographic protein profile of commercial HDL incubated in PBS, in the absence of labeled ANP, yielded only one peak which eluted at the void volume (data not shown), differently from non-incubated HDL (Fig. 4A). Interestingly, aggregated HDL

![Fig. 4. Representative protein profile following gel filtration chromatography of commercial HDL (A). Autoradiography (B) of pooled and concentrated fractions corresponding to molecular mass ranges of $>$1100 kDa (fractions 31–40, pool a), 170–1100 kDa (fractions 41–60, pool b), 56–170 kDa (fractions 61–72, pool c) and 26–56 kDa (fractions 73–80, pool d). See Sections 2.6 and 2.7 for experimental details.](BBADIS 62028 30-5-01)
bearing α-[¹²⁵I]hANP, following incubation in plasma, were partially converted into smaller species (40–170 kDa), suggesting that the peak III material derived from HDL remodeling. It is well known that several plasma factors, including apoproteins, lipolytic enzymes, transfer proteins and lipoproteins acting as lipid acceptors [37–40], are responsible for the remodeling and regulate levels and composition of HDL subclasses. The remodeling hypothesis also gives an explanation for the lack of quantitative correlation between the decrease of peaks I and II and the increase of peak III when HDL is added to plasma. Particularly, the sequestering phenomenon of monomeric ANP by newly generated small HDL particles led to an unproportioned increase in peak III radioactivity. In addition, the increase of peak III when VLDL are added to the plasma now also becomes clear. In fact, it has been demonstrated that the lipoprotein lipase bound to VLDL may facilitate the action of the cholesteryl ester transfer protein by improving the efficiency of VLDL as cholesterol acceptors [41] and thus the remodeling of lipoproteins.

In an attempt to identify the HDL component playing a role in ANP binding we examined the distribution of the protein and lipid constituents among the chromatographic fractions from native and incubated plasma. Just as we expected from plasma incubation, lipid transfer from HDL to other lipoproteins and apolipoprotein redistribution were found. In agreement with what already was reported [37], these processes resulted mainly in the conversion of medium into small HDL. Interestingly, immunoreactive ANP followed the redistribution of apo A-I. In fact, either ANP or apo A-I shifted in the region of small HDL upon plasma incubation. On the basis of this result, we hypothesize an association of ANP and HDL via apo A-I as reported for Aβ [42] and TTR [31].

In addition, we have found that the HDL subclasses display in vitro a different binding capacity for ANP. In fact, the chromatographic profile of bound α-[¹²⁵I]hANP formed in plasma showed that radioactivity is mainly recovered in the fractions including large and small particles of HDL, while only sporadically a binding is observed in the medium HDL region [16]. In contrast, following gel filtration of native plasma, endogenous ANP was also recovered in this latter region. These results are only apparently conflicting. In fact, a recent study [43] states a conversion of small HDL in a unidirectional way into medium or large HDL occurring in a compartment outside the circulating plasma. On the basis of these findings and of the lower binding capacity of medium HDL for ANP, also evidenced by our ligand blotting experiments, we suggest that in vivo small HDL could combine with the hormone in plasma and, following a conversion outside the circulating compartment, return to the plasma pool as medium particles loaded with ANP. In fact, under experimental conditions which do not allow remodeling, like in the ligand blotting procedure, binding of ANP to several HDL particles is observable. At the same time, a maximal binding capacity of ANP was evident for HDL species with a low lipid/protein ratio, thus justifying our major interest in small HDL.

On the basis of the amphipathic nature of both apoproteins [44] and ANP [45], we hypothesize a participation of hydrophobic forces in the interaction of ANP with HDL, as postulated for Aβ [44]. According to the competitive binding between Aβ and lipid to hydrophobic domains of apo E [46], we hypothesize that the differential binding to ANP of small and medium HDL could be the result of the exposure of a larger number of hydrophobic domains in small HDL due to the partial delipidation that occurs during the remodeling process.

Based on the knowledge that the amyloidogenic protein concentration may be critical for the formation of amyloid [3], the biological importance of this interaction might reside in the maintenance of low levels of soluble ANP in biological fluids. In our opinion these studies should be supportive to clinical studies, since no treatment so far exists to specifically resolve the causes of amyloid deposits, while it is clear that all interventions which reduce the supply of the fibril protein precursors should be considered [47].

Acknowledgements

This work was partially supported by a grant from the University of Siena, Italy (Piano di Ateneo per la Ricerca, Esercizio 1999, Quota per Servizi, Area delle Scienze Biomediche e Mediche).
References


