Expression of apolipoprotein[a] and plasminogen mRNAs in cynomolgus monkey liver and extrahepatic tissues

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Abstract The apolipoprotein[a] (apo[a]) and plasminogen (PLG) genes share a high degree of sequence identity, suggesting that both genes may be coordinately expressed. To address this possibility, hepatic apo[a], PLG, and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNAs in 11 cynomolgus monkeys that express plasma Lp[a] over a 10-fold range (5.3–69.3 mg/dl) were measured. This analysis demonstrated a 13-fold variation in apo[a] mRNA. PLG mRNA levels ranged only 3-fold, which was similar to the deviation in G3PDH mRNA expression. Lp[a] and PLG plasma levels were also not related in the animals. To further define expression of the latter mRNAs, they were measured in liver and 13 extrahepatic tissues from 5 monkeys. Apo[a] transcript was detected for the first time in adrenal, lung, and pituitary in addition to brain and testes. PLG mRNA was detected extrahepatically only in testes while G3PDH mRNA was ubiquitously expressed. In individual animals, there was no relationship between hepatic and extrahepatic apo[a] mRNA levels, suggesting tissue-dependent expression of the transcript. These results demonstrate that although the apo[a] and PLG genes are highly homologous, their mRNA expression differs markedly.—**Ramharack, R., M. A. Spahr, J. S. Kreick, and C. S. Sekerke.** Expression of apolipoprotein[a] and plasminogen mRNAs in cynomolgus monkey liver and extrahepatic tissues. *J. Lipid Res.* 1996. **37:** 2029–2040.

Supplementary key words Lp[a] • plasminogen • glyceraldehyde-3-phosphate dehydrogenase • mRNA

Plasma lipoprotein[a] (Lp[a]) levels greater than 20–30 mg/dl are independently associated with a variety of vascular diseases (1–20). The Lp[a] particle is formed extracellularly (21, 22), possibly at the hepatocyte surface (23), through a disulfide bond between apolipoprotein B-100 (apoB-100)-containing lipoproteins and the polymorphic plasminogen-related glycoprotein apolipoprotein[a] (apo[a]). Apo[a] shares an unusually high degree of sequence identity with plasminogen (PLG) (24–26). The apo[a] 5′- and 3′-untranslated regions are 98% and 87% homologous, respectively, to the corresponding sequences in plasminogen mRNA (24). There are also four protein coding domains in apo[a] that share extensive identity (78–100%) with plasminogen. These domains include the leader sequence, kringle IV, kringle V, and the protease region of plasminogen. However, apo[a] does not contain the preactive region and kringles I–III of plasminogen (24). The kringle IV sequence is repeated from 12 to >40 times in apo[a] which distinguishes it from other kringle IV-containing sequences including several apo[a]-related genes (apo[a]-rg) (26). Analysis of an apo[a] cDNA containing 37 kringle IV sequences (24) showed that there are 10 different kringle IV subtypes, designated as types 1–10, that are 78–88% identical to PLG kringle IV at the protein level. The type-1 kringle IV sequence and types 3–10 kringle IV sequences are present as single copies in apo[a] whereas kringle IV, type-2, is the major identically repeated kringle (27) and is responsible for the protein isoform size differences in apo[a] that range from 300 to >800 kDa (28, 29).

The latter sequence data together with the observation that both apo[a] and plasminogen genes are adjacently located on chromosome 6 flanked by apo[a]-rg -B and -C (30) suggest that the apo[a] gene was generated from duplication of an ancestral PLG gene (24). Cloning of part of both the apo[a] and plasminogen 5′-flanking regions demonstrated that these sequences are also highly homologous (25, 26). Transfection of these 5′-flanking regions into HepG2 cells indicated that they had similar transcriptional activity (31) suggesting coordinate expression of the genes. However, analysis of the apo[a] and PLG mRNAs from several organs of a single

Abbreviations: Lp[a], lipoprotein[a]; apo, apolipoprotein; apo[a]-rg, apo[a]-related gene; PLG, plasminogen; MOPS, 3-(n-morpholino) propanesulfonic acid; KIV, kringle IV; G3PDH, glyceraldehyde-3-phosphate dehydrogenase.

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rhesus monkey (32) and a cynomolgus monkey (33) suggested that although the transcripts are synthesized predominantly by liver they are not co-expressed in all extrahepatic tissues. These results suggested that in vivo expression of apo[a] and PLG may be different. Indeed, northern blot analysis of liver RNA isolated from three cynomolgus monkeys demonstrated, qualitatively, that the apo[a] and PLG mRNA were expressed at different levels (33). To more clearly define the relationship between apo[a] and PLG mRNA expression, quantitative ribonuclease protection assays were used to analyze the steady state level of both transcripts and G3PDH mRNA in liver samples and several extrahepatic tissues in a group of cynomolgus monkeys that have plasma Lp[a] levels extending over a 10-fold range.

METHODS

RNA isolation

Tissue samples (adrenal, brain, fat, heart, kidney, liver, lung, pancreas, pituitary, skeletal muscle, small intestine, spleen, stomach, and testes) were dissected from five healthy male cynomolgus monkeys on a normal diet and flash frozen in liquid nitrogen. Liver samples were obtained from an additional six animals. Total RNA was isolated from frozen tissues using RNAzol. Poly(A)$^+$ RNA was prepared using oligodT cellulose spun columns (Clontech).

Probe construction/mRNA analysis

The cynomolgus monkey 342 nucleotide apo[a] identically repeated kringle IV type-2 cDNA was PCR-amplified from a cDNA clone containing the apo[a] 5′-untranslated region, kringle IV type-1 and kringle IV type-2 sequences as described (34). The primers used (20 mers) were based on the human apo[a] cDNA (24) and represent the very 5′- and 3′- ends of the kringle IV type-2 region (nucleotides 435–777 of the human sequence). No PCR products were observed in the absence of input DNA. The amplified fragment was cloned directly into pCRII and three independent clones were sequenced to confirm their identity by comparison to human apo[a] cDNA. Complete agreement was found among the three sequences. These clones were designated pMK-2. A 318 nucleotide antisense riboprobe spanning position 536–777 of the corresponding human apo[a] cDNA and containing 77 nucleotides of pCRII sequence was generated by T7 polymerase transcription of HindIII-digested pMK-2. This probe generated a protected apo[a] mRNA band of 241 nucleotides in a ribonuclease protection assay (see below). A 302 nucleotide sense strand probe, representing nucleotides 436–659 of the human apo[a] cDNA and containing 79 nucleotides of vector sequence was synthesized using T7 polymerase and BamHI linearized plasmid in which the insert was cloned in the reverse orientation relative to pMK-2. Use of the sense strand probe in the ribonuclease protection resulted in the generation of a 123 nucleotide-protected fragment that was used as an internal standard.

A 642 nucleotide region of plasminogen (PLG) mRNA was amplified by RT-PCR of monkey liver total RNA using an upstream primer and a downstream primer that represent nucleotides 291–316 and 912–932, respectively, of the human sequence (35). No RT-PCR products were observed in the absence of RNA. The 642 fragment spans a segment of PLG mRNA containing part of the preactive and kringle III sequence. Its identity was confirmed by sequence analysis and it was designated pMPLG. In the PLG mRNA ribonuclease protection assay, a 754 nucleotide antisense riboprobe generates a 642 nucleotide-protected mRNA fragment that represents the entire size of the PLG cDNA insert and a 754 nucleotide sense strand probe generates a 656 nucleotide PLG internal standard.

Monkey liver glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA was RT-PCR amplified using commercial primers (Clontech) that span nucleotides 586–1036 of the human cDNA, cloned directly into pCRII, and sequenced to confirm its identity. This clone was designated pMG3. No RT-PCR products were observed in the absence of input RNA. In the G3PDH mRNA ribonuclease protection assay, a 543 nucleotide antisense riboprobe generates a 450 nucleotide-protected mRNA fragment that represented the complete pMG3 cDNA insert and a 558 nucleotide sense strand probe generated a 506 nucleotide G3PDH internal standard.

A solution hybridization was used to measure apo[a], PLG, and G3PDH mRNAs using the probes described above. Approximately 100 pg of ^{32}P-labeled anti-sense probe, 10 pg of sense-strand internal standard, and total monkey RNA (10–50 μg) or yeast t-RNA (50 μg) were added to a 0.5 ml GeneAmp thin-walled (Perkin-Elmer) reaction tube and dried down using a SpeedVac. The resulting pellet was completely dissolved in 4 μl buffer consisting of 0.4 M NaCl, 1 mM EDTA, and 40 mM MOPS, pH 7.0, followed by addition of 16 μl of deionized formamide. The tubes were placed in a Perkin-Elmer 9600 thermocycler and the following program was used for one cycle: 85°C for 15 min and 45°C for 16 h. Tubes were removed from the thermocycler and 280 μl of a solution containing 1.5 M NaCl, 25 mM EDTA, 50 mM Tris-HCl, pH 7.5, 0.05 mg/ml ribonuclease AI, and 0.2 units of ribonuclease TI was immediately added and incubated at 30°C for 1 h. After the nuclease digestion, samples were adjusted to 0.7% SDS and 0.17 mg/ml

proteinaseK and incubated at 37°C for 15 min. This reaction was terminated by extraction with an equal volume of phenol–chloroform–isoamly alcohol 25:24:1. The upper phase was removed to a clean tube containing 20 μg of yeast t-RNA and ethanol precipitated. The resulting pellet was washed with 75% ethanol, resuspended in 4 μl of loading buffer (90% deionized formamide, 20 mM EDTA, pH 8.0, 0.02% bromophenol blue, and 0.02% xylene cyanol) and heated at 70°C for 10 min. The heated samples were immediately loaded onto 6% sequencing gels and electrophoresis was carried out at constant power (80 watts) until the bromophenol blue migrated to the gel bottom. Gels were transferred to developed X-ray film, covered with plastic wrap, and exposed to a storage phosphor screen (Molecular Dynamics) overnight. Exposed screens were scanned using a PhosphorImager and the resulting bands were quantitated using ImageQuant software (Molecular Dynamics).

Northern blots were carried out as detailed previously (34). Gels and blots were analyzed using a PhosphorImager and ImageQuant software.

Lipoprotein/apolipoprotein analysis

Monkey plasma Lp[a] was assayed as previously described (34) using a commercially available ELISA (Apo-Tek) that measures total Lp[a] mass. This ELISA uses a monoclonal anti-human Lp[a] capture antibody (2D1) directed towards the unique protease domain of apo[a] and a horseradish peroxidase-labeled polyclonal sheep anti-human apoB-100 detection antibody (36); consequently, the assay is unaffected by apo[a] isoform size and dose not detect "free" apo[a] (we are unable to detect "free" apo[a] in monkey plasma by western blot analysis). The Lp[a] ELISA does not detect plasminogen or apoB-100 (36).

Apo[a] isoform sizes were determined on 10 mM DTT reduced plasma samples by western blots using a Lp[a]/apo[a] polyclonal antibody (Biodesign). Samples were resolved on 4%, Tris-glycine, polyacrylamide mini gels (Novex) at 140 volts for 2.5 h at room temperature. Human apo[a] molecular weight standards, kindly provided by Drs. Joel Morrisett and John Gaubatz (Baylor College of Medicine, Houston, Texas), were used to determine the apo[a] isoform sizes in the monkeys. These standards had molecular masses of 400, 525, 600, 700, and 800 kDa.

Plasminogen assay

Plasminogen was assayed in monkey plasma using a commercially available kit and reference plasma (Sigma). In this assay, excess streptokinase is added to plasma which forms a plasminogen–streptokinase complex that possesses plasmin-like activity. The plasmin activity is determined using a plasmin-specific chromogenic substrate. The amount of amidolytic activity generated is directly proportional to the amount of plasminogen in the sample. This assay is not sensitive to plasma inhibitors. Plasminogen levels are expressed as a percentage of the reference human plasma which is set at 100% PLG. Pooled plasma from a group of 10 cynomolgus monkeys had plasminogen levels that were 14% of the reference plasma (14% PLG). This 14% PLG level was used as the 100% value for monkey plasminogen.

RESULTS

The liver is the major site of both apo[a] and PLG mRNA synthesis (32, 33), therefore we decided to examine the relationship between hepatic apo[a] and PLG mRNA levels. Both transcripts were assayed in total liver RNA isolated from several animals that had very different plasma Lp[a] levels (range 5.3–69.6 mg/dl). Monkey apo[a] mRNA levels in these experiments were determined by a specific ribonuclease protection assay that uses a kringle IV type-1 probe as previously characterized (34). In the latter assay, apo[a] mRNA measurements are unaffected by the number of kringle IV repeats, therefore, the protected band is directly proportional to the apo[a] mRNA abundance. PLG mRNA levels were also determined by ribonuclease protection assay using a cloned monkey cDNA. The PLG probe spanned part of the preactive domain, kringles I and II and part of kringle III, consequently, this probe will not cross-react with apo[a] mRNA. G3PDH mRNA was measured by a ribonuclease protection assay in all samples as a control. (Representative examples of the PLG and G3PDH ribonuclease protection assays are shown in Figs. 7 and 8, respectively). The PLG mRNA band runs as a closely spaced doublet in the PLG ribonuclease protection assay (see Figs. 7A and 7B). This doublet pattern may be due a ribonuclease hypersensitive site in the PLG mRNA protected fragment or the result of an alternatively spliced PLG mRNA. However, northern blots demonstrated (data not shown) that the PLG probe hybridizes to a single mRNA of 2.7 kb which may rule out the possibility of alternatively spliced PLG transcripts.

The apo[a] and PLG mRNA levels normalized to G3PDH transcript levels and plotted against their corresponding plasma protein concentrations are shown in **Figs. 1A and 1B,** respectively. This analysis indicated that liver apo[a] mRNA and plasma Lp[a] varied approximately 20-fold and 13-fold, respectively (Fig. 1A). In contrast, PLG mRNA and plasma PLG had a much narrower range of values that differed only 3-fold and 2.9-fold, respectively (Fig. 1B). The magnitude of the

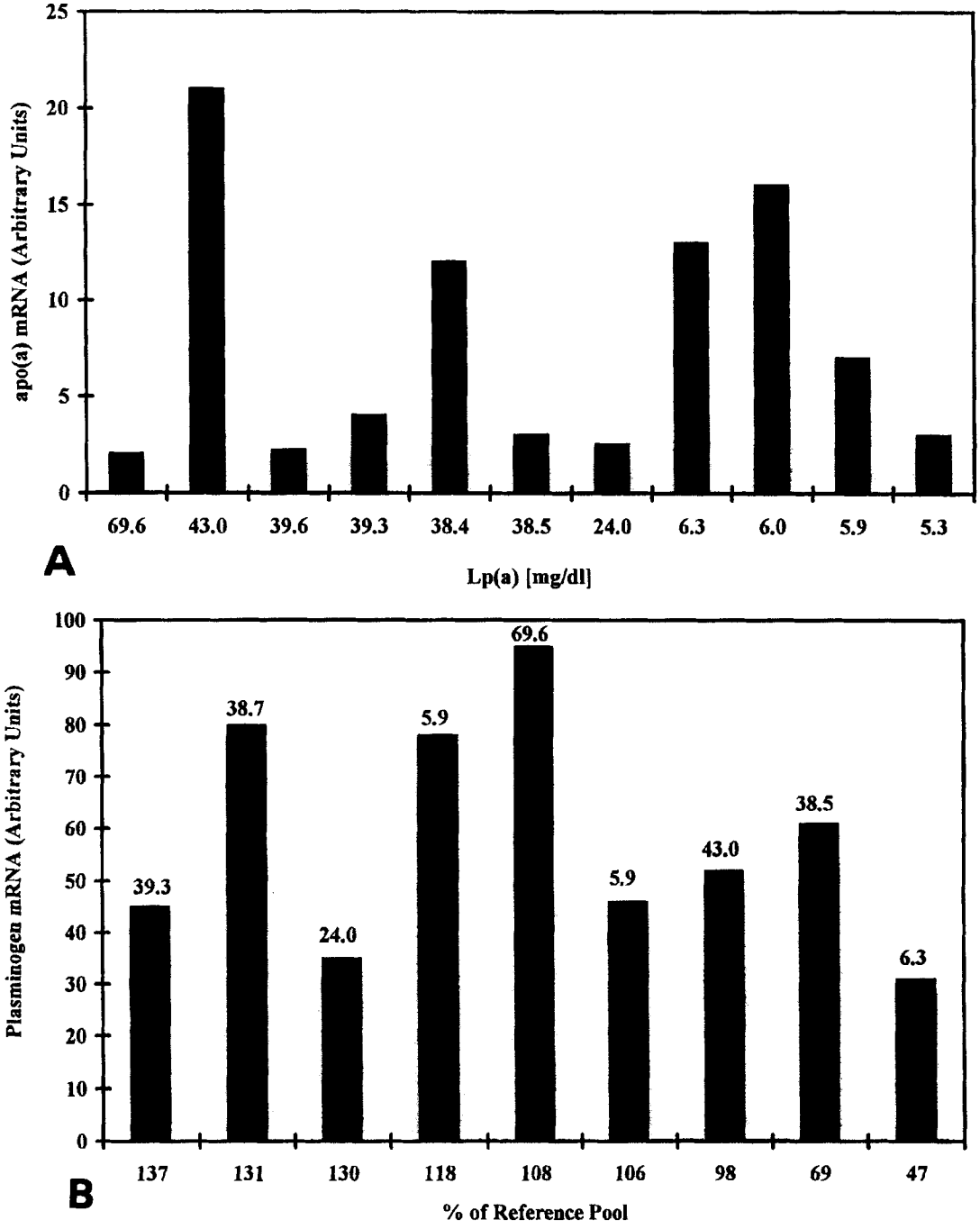

Fig. 1. A: Monkey liver apo[a] mRNA and plasma Lp[a] levels. Monkey liver apo[a] mRNA was determined by a ribonuclease protection assay using an apo[a] kringle IV type-1 probe as described in Materials and Methods. The apo[a] mRNA levels were normalized to G3PDH mRNA expression which was also measured by a ribonuclease protection assay described in Materials and Methods. Plasma Lp[a] levels were assayed by a sandwich ELISA that is not influenced by apo[a] isoform size, apoB-100, or PLG. The 11 animals used had plasma Lp[a] levels of 5.3–69.3 mg/dl. B: Monkey liver plasminogen mRNA and plasma plasminogen levels. Plasminogen (PLG) mRNA was measured using a ribonuclease protection assay as described in Materials and Methods in the same group of 11 animals used to analyze apo[a] mRNA and Lp[a]. PLG mRNA levels were normalized to G3PDH transcript levels as described in Fig. 1A. Plasma PLG was determined as described in Materials and Methods using a chromogenic assay. The animals in which both liver PLG mRNA and plasma PLG were determined are shown, together with their corresponding Lp[a] levels (mg/dl) displayed above the bars.

differences in PLG mRNA observed in the individual animals was similar to that seen with the G3PDH mRNA control, indicating that the PLG mRNA differences represented a normal variation amongst the animals.

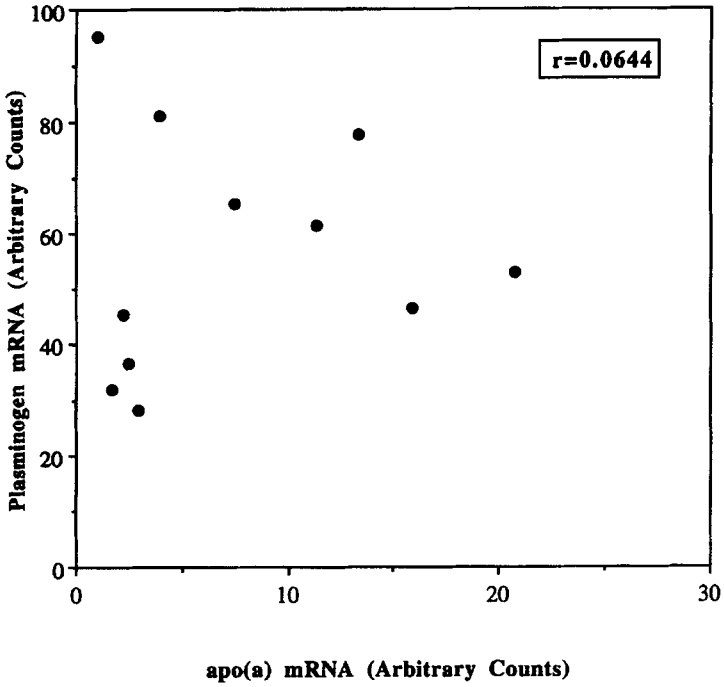

Fig. 2. Regression analysis of hepatic apo[a] and PLG mRNA levels. Liver apo[a] and PLG mRNA levels from the 11 monkeys described in Fig. 1A and 1B were analyzed to determine the relationship between their expression.

To determine whether there was a correlation between the apo[a] and PLG mRNA expression in the 11 animals, a regression analysis was carried out on the mRNA data. This showed that there was no correlation between apo[a] and PLG mRNA levels (r = 0.0644, **Fig. 2**). There was also no relationship between plasma Lp[a] and PLG (r = 0.213).

Apo[a] isoform sizes were determine in the monkeys by western blot analysis to define the relationship between apo[a] mRNA abundance and apo[a] isoform size. A plot of apo[a] transcript abundance versus apo[a] isoform size is shown in **Fig. 3.** Regression analysis of this data demonstrated no relationship between apo[a] mRNA levels and apo[a] molecular weight (r = 0.0396), but, there was an inverse relationship between apo[a] isoform size and plasma Lp[a] levels (r = -0.78416). Although, there appeared to be a negative correlation between Lp[a] and apo[a] mRNA levels (Fig. 1A), regression analysis of the data showed their concentration to be unrelated (r = -0.3128).

To further characterize expression of the apo[a] and PLG mRNAs, their tissue distribution along with that of

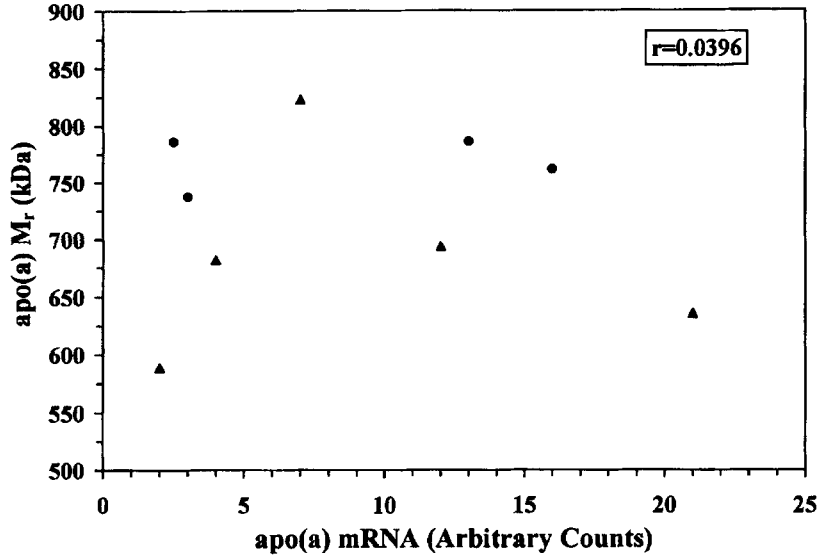

Fig. 3. Relationship between apo[a] isoform size and hepatic apo[a] mRNA levels. Apo[a] isoform size was determined by western blots on 10 mM DTT reduced plasma samples from the monkeys described in Fig. 1. Monkey apo[a] isoform sizes were measured by comparison to human apo[a] standards as described in Materials and Methods. Regression analysis of apo[a] isoform size versus apo[a] mRNA levels in nine animals is shown. M_r, molecular weight; ▲, single apo[a] isoform animals; ●, double apo[a] isoform animals (average M_r).

G3PDH mRNA was determined in 13 extrahepatic tissues of five healthy monkeys. Initial attempts to measure extrahepatic expression of apo[a] mRNA using the kringle IV type-1 probe proved unsuccessful, probably as a consequence of the low apo[a] transcript expression in these tissues. To circumvent this problem, a ribonuclease protection assay was developed using the identically repeated cynomolgus monkey kringle IV (type-2) cDNA sequence. The type-2 kringle IV sequence is repeated a variable number of times in apo[a] mRNA depending on its isoform size (27, 28). A probe that hybridizes to this region will result in an amplification of the signal that is proportional to both the number of kringle IV type-2 repeats in a particular apo[a] transcript and its abundance, thereby, allowing for a very sensitive measure of the apo[a] mRNA molecule. Alignment of the kringle IV type-2 probe sequence to the entire GenBank and EMBL nucleotide data bases demonstrated that only the apo[a] kringle IV type-2 sequence was 100% identical to the probe region. All other matched sequences including other apo[a] and apo[a]-rg kringle IV sequences showed stretches of mismatched nucleotides that would result in substantial reductions in the expected size of the apo[a] protected fragment generated using the kringle IV type-2 probe. To confirm the functional specificity of the kringle IV type-2 probe in detecting apo[a] mRNA, northern blot analysis of monkey liver total RNA and polyadenylated RNA was performed. These experiments showed that the type-2 probe hybridized to a single high molecular weight mRNA species of 10.3 kb in both total and polyadenylated liver RNA under stringent hybridization and washing conditions (**Fig. 4**) that was consistent with the apo[a] protein isoform size in this animal (~500 kDa).

The probe described above was used to examine apo[a] mRNA expression in adrenal, brain, fat, heart, kidney, liver, lung, pancreas, pituitary, skeletal muscle, small intestine, spleen, stomach, and testes tissue of five

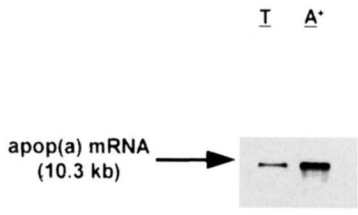

Fig. 4. Northern blot analysis of monkey apo[a] mRNA. Total (10 μg) or polyadenylated (1 μg) liver RNA from a monkey expressing a single ~500 kDa apo[a] isoform was denatured with formaldehyde, resolved on 1% agarose gels, and transferred to Nylon-1 membranes as described in Materials and Methods. The blot was probed with nick-translated purified monkey kringle VI type-2 cDNA labeled with ^{32}P and washed under stringent conditions. After washing, the blot was exposed to a PhosphorImager screen overnight. T, total liver RNA; A+, polyadenylated liver RNA.

healthy male cynomolgus monkeys on a normal chow diet. Three criteria were used to determine whether a particular tissue expressed apo [a] mRNA when using the ribonuclease protection assay: *1)* the protected band had to be of the predicted size; *2)* the band had to be absent from the yeast control and; *3)* the band had to be present in all five animals (plasma Lp[a] was expressed in all animals used in the study). **Figure 5** is a representative example of the tissue distribution of apo[a] mRNA seen in the five monkeys. The liver was found to express the highest levels of apo[a] mRNA, a finding consistent with previous studies (32, 33). Apo[a] mRNA expression was found in the testes and brain, which had also been documented earlier in the rhesus monkey (32). The most surprising finding was apo[a] mRNA detection in lung, adrenal, and pituitary which is reported here for the first time.

Relative to liver, extrahepatic apo[a] mRNA levels were highest in the brain and lowest in the adrenal (**Table 1**). No apo[a] mRNA expression was detected in all other tissues examined. Comparison of the liver apo[a] mRNA concentration to that of extrahepatic tissues containing the apo[a] transcript indicated that there was no relationship between the hepatic and nonhepatic apo[a] mRNA levels (**Fig. 6**).

PLG mRNA was measured by a ribonuclease protection assay in the same RNA samples used for apo[a] mRNA analysis. The PLG ribonuclease protection assay detects PLG mRNA as closely spaced doublets, below the PLG standard, as readily seen in liver samples (**Fig. 7A**). In extrahepatic tissues, the bands observed below the PLG Std. do not co-migrate with the PLG mRNA doublet observed in liver (Fig. 7A), consequently, they represent degraded probe intermediates such as those seen in other parts of the gel and not PLG mRNA. The relatively constant intensity of these bands and their presence in samples containing only yeast t-RNA (data not shown) also argue that they are the result of probe degradation and not authentic PLG mRNA. This pattern of PLG mRNA expression was seen in all 13 tissues from the five monkeys. However, longer exposure of the PLG ribonuclease protection gels revealed traces of bands, that were the same size as the liver PLG mRNA bands, in testes but not in other extrahepatic tissue. Expression of PLG mRNA in the testes was confirmed by reassaying larger amounts of testicular total RNA samples from three monkeys (Fig. 7B). Fat total RNA was also reanalyzed and found not to contain PLG mRNA as observed in Fig. 7A. In this experiment, the smearing below the PLG Std. in the fat lane (Fig. 7B) does not co-migrate with the PLG mRNA and represents degraded probe intermediates as discussed above.

The lack of apo[a] and PLG expression in certain tissues such as muscle and stomach may have been the result of

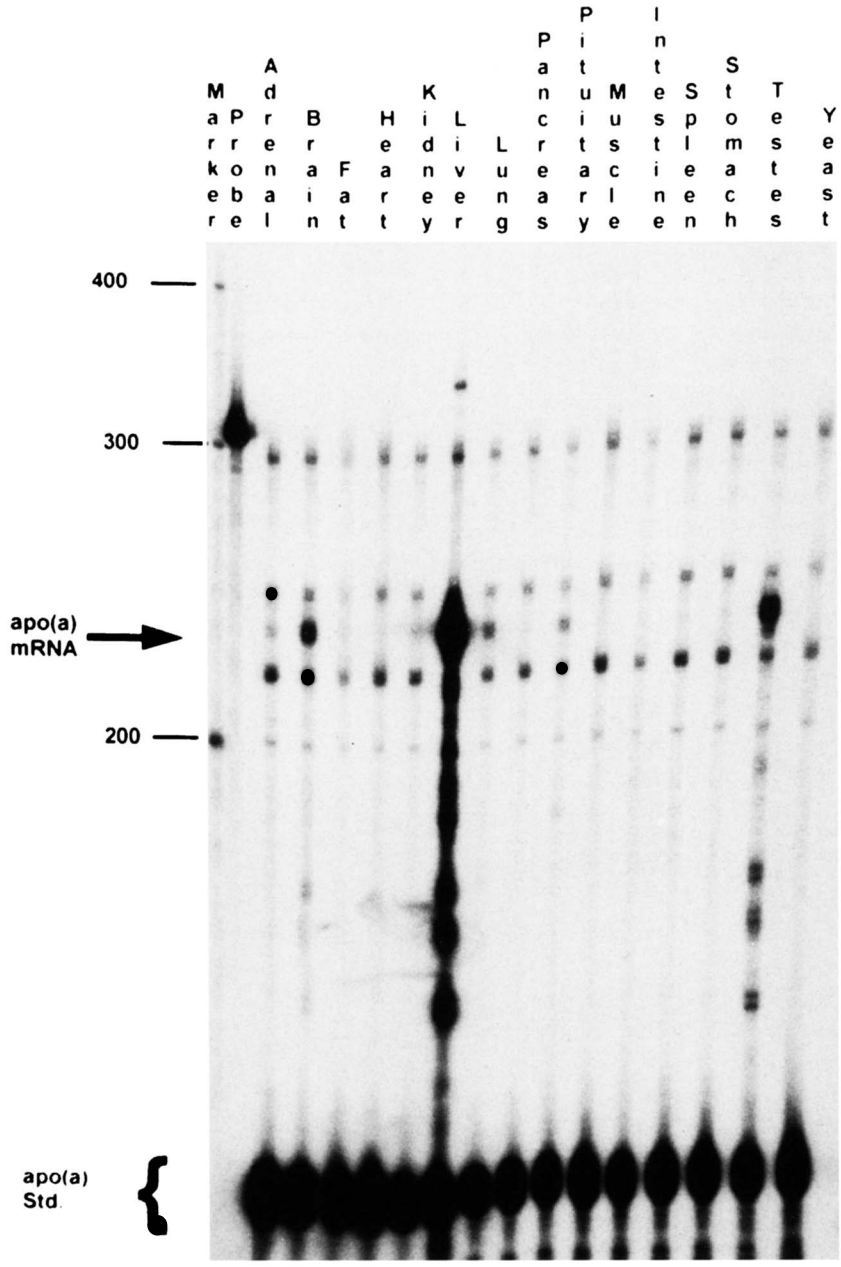

Fig. 5. Representative example of apo[a] mRNA tissue distribution. Apo[a] mRNA levels were determined in total RNA (30 μg) extracted from liver and 13 extrahepatic tissues of five monkeys by a ribonuclease protection assay that uses a kringle IV type-2 probe. The figure is a representative example of the apo[a] mRNA tissue distribution from one animal. Samples are arranged in alphabetical order starting with adrenal and ending with testes; muscle, skeletal muscle; intestine, small intestine; probe, 302 nucleotides; apo[a] mRNA, 241 nucleotides; apo[a] Std., apo[a] internal control standard, 123 nucleotides. The apparent apo[a] mRNA band in the kidney RNA lane is due to bleeding of the intense apo[a] mRNA band in the adjacent liver RNA track and is not due to the presence of apo[a] mRNA in the kidney samples. No apo[a] mRNA was found in the other four monkey kidney samples or when the kidney total RNA used in this figure was re-assayed.

sample degradation preventing the measurement of the mRNAs in these samples. This was addressed by analyzing G3PDH mRNA expression in all the tissue samples (**Fig. 8**). The G3PDH transcript was present in every sample indicating that total RNA isolated from the various tissues were intact. Furthermore, in liver, where apo[a] and PLG mRNAs are expressed at their highest levels, G3PDH mRNA concentrations are lower than several organs (e.g., heart, kidney, skeletal muscle, Fig. 8) that did not contain either apo[a] or PLG transcripts.

TABLE 1. Summary of apo[a] mRNA extrahepatic distribution

Extrahepatic Tissue	Apo[a] mRNA
	%
Adrenal	0.41 ± 0.23
Brain	2.88 ± 0.67
Lung	1.43 ± 0.36
Pituitary	0.60 ± 0.28
Testes	2.32 ± 0.59

The average apo[a] mRNA distribution in extrahepatic tissues of the five monkeys is displayed as a percent of liver levels ± SD. Apo[a] mRNA levels were determined by ribonuclease protection assay as described in Fig. 5.

DISCUSSION

Apo[a] is believed to have arisen by multiple duplication of the plasminogen gene resulting in a high degree of sequence homology between the coding, non-coding and 5′-flanking regions of both sequences (24–26). The near identity in the first 700 nucleotides of 5′-flanking region between the two genes and the similar transcriptional activity of reporter gene constructs containing this region when they are transfected into HepG2 cells suggests that the apo[a] and PLG genes may be coordinately expressed (31). In addition, a HNF-1α footprint in the apo[a] promoter (+47 to +26) that is responsible for the basal activity of 1.4 kb of apo[a] 5′-flanking region is also present in the same region of the PLG promoter (31), further suggesting that similar factors control both genes. However, in the monkeys used in this study, there was no correlation between apo[a] and PLG liver mRNA steady state levels or plasma Lp[a] and PLG concentrations, suggesting independent expression of the molecules at both the transcript and protein levels. The tissue distribution studies further support the finding that both genes are differently expressed by demonstrating a much wider extrahepatic dispersion of apo[a] mRNA than PLG mRNA. Collectively, the data argue that al-

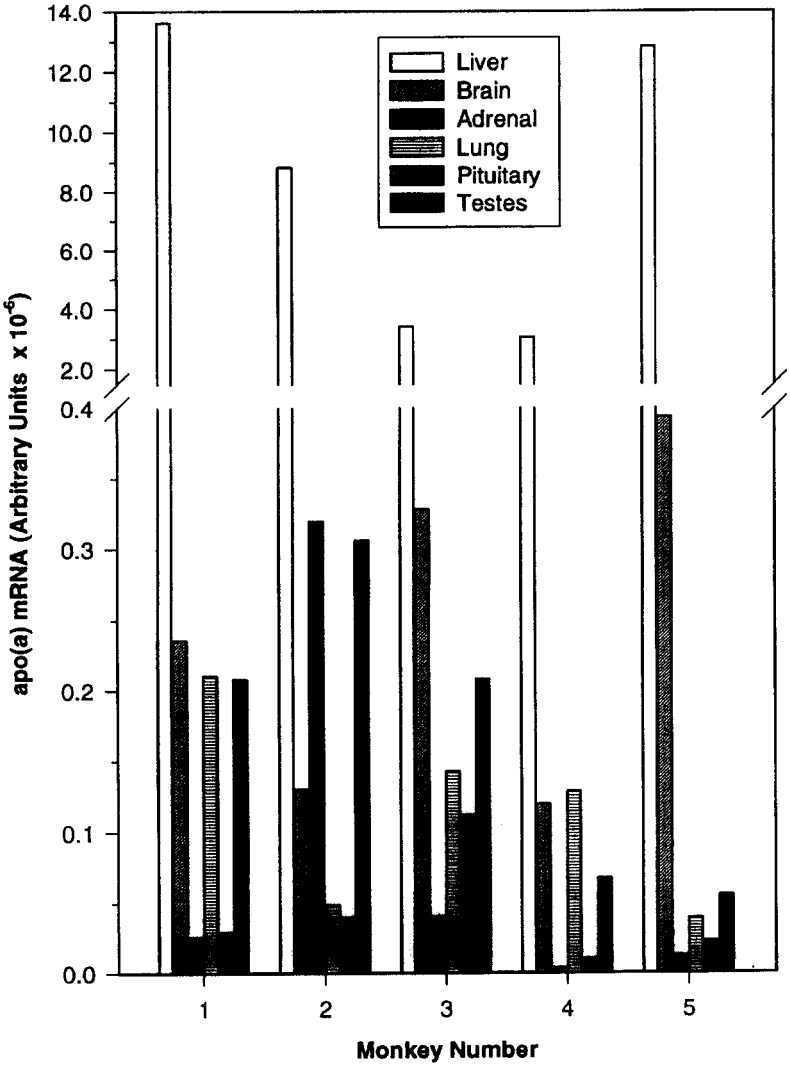

Fig. 6. Comparison of hepatic and extrahepatic apo[a] mRNA distribution in individual monkeys. Liver, brain, adrenal, lung, pituitary, and testes apo[a] mRNA levels in the five monkeys used in this study are shown. Apo[a] mRNA levels were determined in the various tissues as described in Fig. 5.

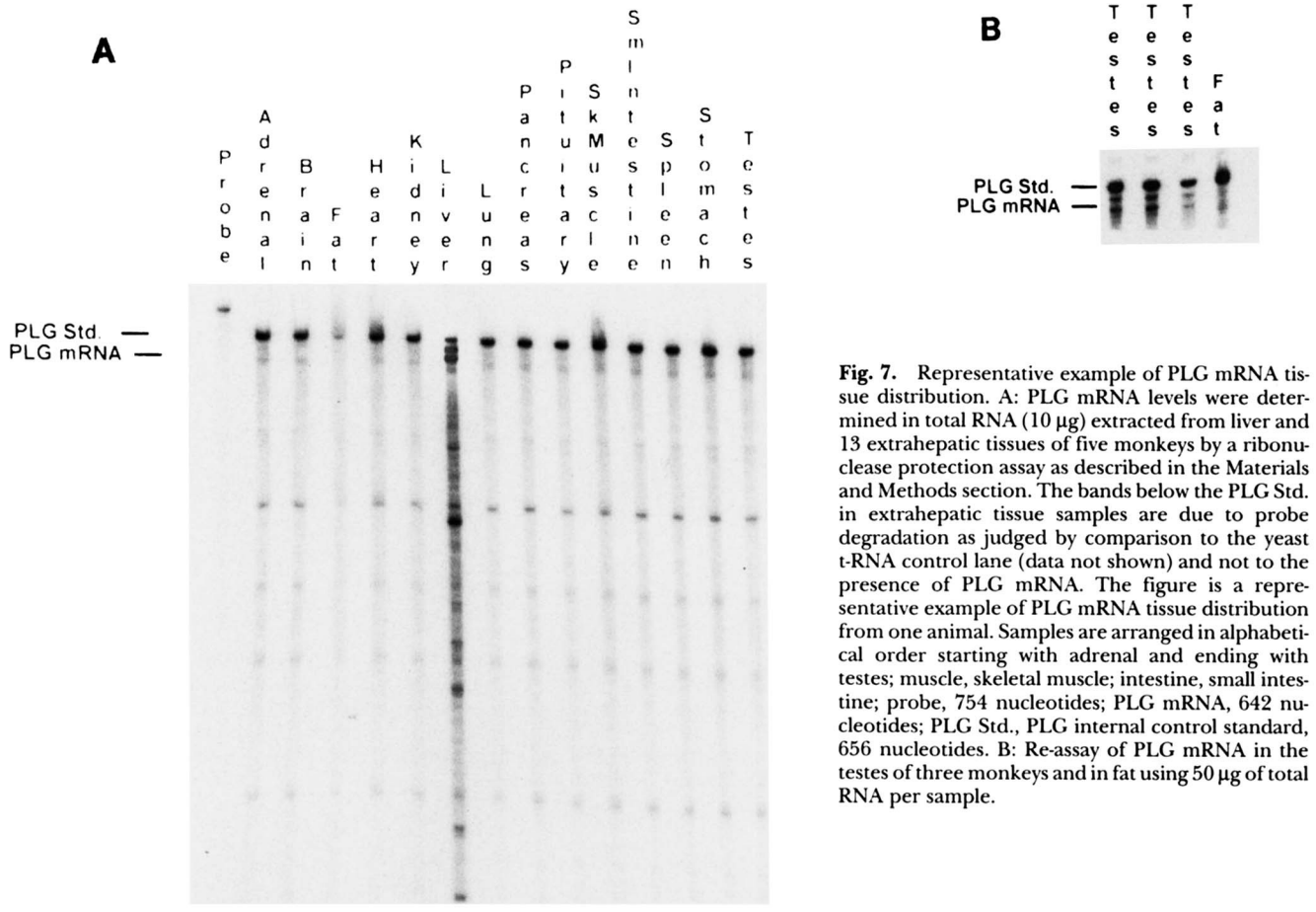

Fig. 7. Representative example of PLG mRNA tissue distribution. A: PLG mRNA levels were determined in total RNA (10 μg) extracted from liver and 13 extrahepatic tissues of five monkeys by a ribonuclease protection assay as described in the Materials and Methods section. The bands below the PLG Std. in extrahepatic tissue samples are due to probe degradation as judged by comparison to the yeast t-RNA control lane (data not shown) and not to the presence of PLG mRNA. The figure is a representative example of PLG mRNA tissue distribution from one animal. Samples are arranged in alphabetical order starting with adrenal and ending with testes; muscle, skeletal muscle; intestine, small intestine; probe, 754 nucleotides; PLG mRNA, 642 nucleotides; PLG Std., PLG internal control standard, 656 nucleotides. B: Re-assay of PLG mRNA in the testes of three monkeys and in fat using 50 μg of total RNA per sample.

though the first approximately 700 nucleotides of apo[a] and PLG 5′-flanking region are closely related (25, 26) this region may be responsible for only minimal promoter activity and that regulatory elements responsible for the major transcriptional control of the two genes lie outside of this region of shared homology. In this regard, beyond position -711 there is no homology between the two sequences (25). Expression of the apo[a] and PLG genes may be analogous to α-fetoprotein and serum albumin expression in which elements controlling their full transcriptional activity are located 2.5–10 kb upstream of their respective transcription initiation site (37–40).

In these studies, we found no correlation between hepatic apo[a] mRNA abundance and plasma Lp[a] levels. This finding contrasts with previous work showing a small but significant correlation between apo[a] transcript and Lp[a] protein in cynomolgus monkeys (33). The reasons for the differences between the two studies are not readily apparent. Previous investigations have demonstrated that several post-transcriptional factors are involved in governing plasma Lp[a] levels such as apo[a] isoform size (33, 41), processing of the nascent apo[a] protein (42), and LDL composition (43). Therefore, apo[a] mRNA abundance may be only one of several factors responsible for governing plasma Lp[a] concentrations. Consequently, apo[a] mRNA abundance and plasma Lp[a] levels may not necessarily be directly related.

This study documents a strong negative correlation between plasma Lp[a] levels and apo[a] isoform size (r = -0.784) as found by other investigators (41). However, we describe for the first time, a lack of correlation between apo[a] isoform size and hepatic apo[a] mRNA abundance. This observation is consistent with another study demonstrating no relationship between apo[a] mRNA abundance and apo[a] mRNA length (33). These observations suggest that unique promoter elements in individuals may control expression of apo[a] mRNA independent of the size of the apo[a] allele.

We have also demonstrated apo[a] mRNA expression in adrenal, lung, and pituitary. These new findings document a much wider extrahepatic expression of apo[a] mRNA than previously assumed (brain and testes). Furthermore, comparison of hepatic and extrahepatic apo[a] mRNA in individual monkeys demonstrated no relationship between their levels. This observation suggests that expression of hepatic and

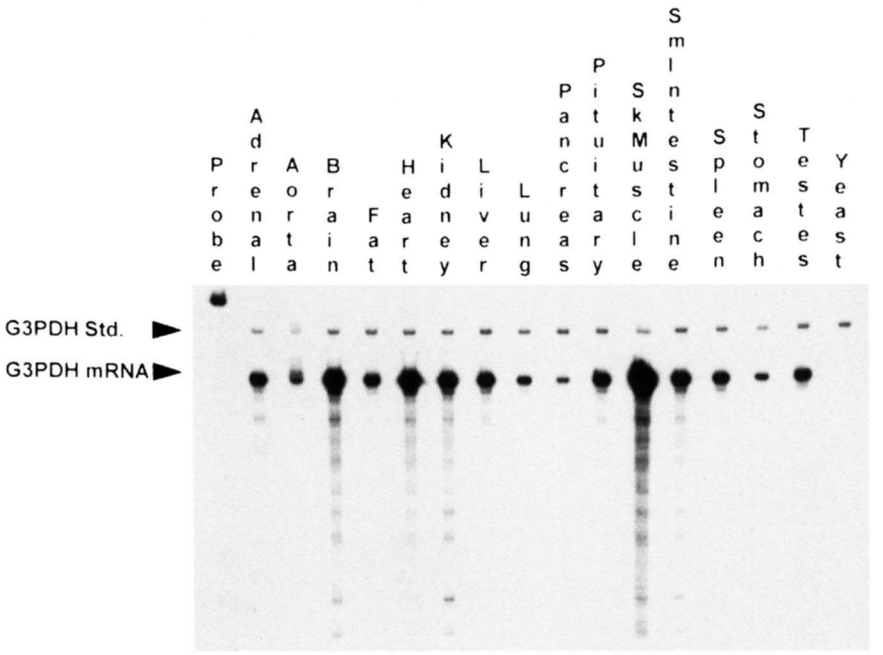

Fig. 8. Representative example of G3PDH mRNA tissue distribution. G3PDH mRNA levels were determined in total RNA (10 μg) extracted from liver and 13 extrahepatic tissues of five monkeys by a ribonuclease protection assay as described in Material and Methods. The figure is a representative example of the G3PDH mRNA tissue distribution from one animal. Samples are arranged in alphabetical order starting with adrenal and ending with testes; muscle, skeletal muscle; intestine, small intestine; probe, 543 nucleotides; G3PDH mRNA, 450 nucleotides; G3PDH Std., G3PDH internal control standard, 506 nucleotides.

extrahepatic apo[a] mRNA levels are under separate control mechanisms which may indicate different roles for apo[a] in liver versus peripheral tissue.

The lung, adrenal, and pituitary can freely exchange components with the blood, suggesting they may contribute to plasma Lp[a] levels by providing a source of secreted apo[a] that can couple to apoB-100-containing lipoproteins in plasma. However, the low apo[a] mRNA expression in these tissues, relative to liver, makes it unlikely that their contribution to plasma Lp[a] would be of major significance. Instead, apo[a] produced in these extrahepatic tissues may play a local role in lipid transport and/or metabolism. It is tempting to speculate that extrahepatic apo[a] serves as an additional means by which cells can extract and internalize apoB-100-containing lipoproteins from the circulation for their local use. This pathway may become more relevant in situations where the LDL receptor, which does not clear apo[a]/Lp[a] (44), is down-regulated by providing an independent means for lipoprotein uptake, thus preventing possible deleterious cellular affects associated with lipoprotein depletion. Apolipoprotein E which is expressed in many extrahepatic tissues including the lung, adrenal, testes, and brain (45, 46) has also been proposed to take part in lipoprotein transport in extrahepatic tissues. Additionally, apo[a] has been shown to decrease the activity of TGF-β by attenuating its activation (47). Inhibition of TGF-β can result in smooth muscle cell proliferation and migration at the site of vascular injury leading to detrimental occlusion of the damaged site (48). This sequence of events may explain the role of Lp[a] in restenosis after balloon angioplasty (19, 20, 49). Therefore, apo[a] can have a profound local role in cellular metabolism because of its potential ability to modulate the activity of cytokines and possibly other regulatory molecules such as hormones. These regional roles for apo[a] would help explain its presence in brain and testes, tissues separated from the circulation by blood–organ barriers making it unlikely that apo[a] produced at these sites would be secreted into plasma for Lp[a] particle formation.

The presence of apo[a] mRNA in the extrahepatic organs is good evidence that apo[a] protein is synthesized at these sites. Nevertheless, the low level of extrahepatic apo[a] mRNA expression (Table 1) raised the possibility that the apo[a] transcript found at these sites was derived from blood cells such as monocytes that had invaded the various organs. However, apo[a] mRNA was not present in spleen which contains approximately 0.6% of its cells as macrophages (46) making it unlikely that the apo[a] transcript is derived from these circulating cells. Furthermore, other tissues such as the brain and testes, which are unlikely to contain resident macrophages, express significantly more apo[a] mRNA than lung which has a high probability of containing these cells.

Our data show no coordination between apo[a] and PLG mRNA expression in cynomolgus monkey. This observation suggests it may be possible to modulate Lp[a] without effecting PLG levels, thereby potentially reducing the risk of vascular disease associated with elevated Lp[a] without having a detrimental plasminogen lowering effect.

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