Albumin obscures sex differences in blood protein patterns of rats and humans

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(Received 4 September 1980/Accepted 5 September 1980)

Sex related differences in the blood protein patterns of male and female rats and humans have been studied by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. In rats, a prominent band of mol.wt. 74 000–78 000 is seen in females in far greater quantity than in males, castrated males or ovariectomized females. A secondary band of 100 000 is seen under non-reducing conditions in female rats that is absent in males. In humans, bands of 92 000 and 88 000 mol.wt. appear to be variable in concentration in men although relatively constant in women. The above differences are observable only if serum albumin is removed from the samples before electrophoresis. The results suggest that each sex has its own characteristic blood protein pattern.

In most species females outlive males (Hamilton, 1948). With this realization has come the concomitant observation that males tend to develop cardiovascular disease at earlier ages than females (Kannel *et al.*, 1979). Consequently, there have been studies in the past, aimed at delineating physiological and biochemical differences between males and females (Haynes & Feinleib, 1980). Of particular interest have been studies measuring levels and classes of lipoproteins such as HDL and LDL due to their putative connection with cardiovascular diseases (Castelli *et al.*, 1977). These studies have concentrated mainly on specific classes and subclasses of molecules and little attention has been paid to the overall pattern of circulating proteins. We sought, therefore, to begin to compare the patterns of circulating proteins in rats and humans of both sexes.

Materials and methods

Blood samples

Blood was taken by closed-chest cardiac puncture from 4-month-old Sprague–Dawley or Wistar rats, with disodium EDTA as the anticoagulant. Plasma was removed by centrifugation. Human serum was obtained by venipuncture from healthy donors between the ages of 18 and 30, of the phenotype A Rh+.

Abbreviations used: HDL, high-density lipoprotein; LDL, low-density lipoprotein; SDS, sodium dodecyl sulphate; VLDL, very-low-density lipoprotein.

Albumin removal

Albumin was removed from serum or plasma by affinity chromatography by using Cibacron Blue-Sepharose (Pharmacia). The method was essentially that of Travis & Pannel (1973) with the following modifications. For rat blood, the column was equilibrated with 0.1 M-NaCl/0.05 M-Tris base (pH 8.0) and for human blood, with 0.1 M-KCl/0.05 M-Tris acid (pH 7.0) (Mahany *et al.*, 1980).

Polyacrylamide-gel electrophoresis

Polyacrylamide-gel electrophoresis was performed under reducing or non-reducing conditions, essentially by the method of Laemmli (1970). Both single-density and linear-gradient gels were used. Proteins were visualized by staining with Coomassie Brilliant Blue R-250 (Wilson, 1979). Protein concentrations were determined by the method of Lowry *et al.* (1951), with fraction V bovine serum albumin as the standard (50 µg applied to each lane). Molecular-weight markers were purchased from Pharmacia Fine Chemicals.

Computerized densitometry

Relative concentrations of the 88 000 and 92 000 mol.wt. bands were determined by computerized densitometry by using the TEXAC system (Ledley *et al.*, 1978). Briefly, the gel photograph is converted into a digital image of 512 × 640 points (pixels), each pixel representing the area of a circle with a diameter of 0.015 inch. Each pixel is stored in the memory in 8 bits, thus the gray level of

each pixel can be assigned a value from 1 to 256. The relative concentrations of the bands in question are determined by summing the gray levels of all pixels within the boundaries of each band.

Results and discussion

We first analysed whole serum from men and women [Fig. 1(a)] and whole plasma of rats [Fig. 1(b)] by SDS/polyacrylamide-gel electrophoresis under reducing conditions. No differences between men and women or among male, female, castrated male and castrated female rats were apparent. The major band, M_r 67000, is serum albumin that constitutes 50–60% of the total protein in the circulation (Young & Webb, 1978). Albumin was therefore removed before SDS/polyacrylamide-gel electrophoresis to assess more subtle differences between the sexes.

Fig. 2 represents a typical experiment in which the plasma minus albumin fractions are compared under reducing conditions, among intact, ovariectomized and castrated rats. The most striking feature is the presence of a band in intact females of approx. 74000–78000 mol.wt. This band appears to be present in considerably reduced quantity in intact and castrated males, as well as castrated females. A secondary band of approx. 50000 is present in greater amounts in the ovariectomized female, relative to the other three groups. The most prominent band in both intact and castrated males has an apparent mol.wt. of 180000, whereas the most prominent band of the intact female is approx. 82000. The amount of the 180000 band appears to increase relative to the 82000 band in ovariectomized female rats.

To further define the nature of these differences in rodents, SDS/polyacrylamide-gel electrophoresis was performed without reduction of disulphide bonds by mercaptoethanol. In Fig. 3 the 74000–78000 band of Fig. 2 is still present in considerably greater quantity in females than in males. Since it is present at the same molecular weight with and without mercaptoethanol reduction, this protein probably exists in the circulation as a single chain. The other noteworthy features of this gel pattern are: (i) the presence of a band of approx. 100000 in females, that is absent in males, and (ii) apparent female elevations in the amounts of two minor bands corresponding to 63000 and 54000. Positive identification of these proteins has not yet been attempted.

A proline-rich lipoprotein associated with VLDL and chylomicrons has been shown to have a mol.wt. of 74000 in humans (Sata *et al.*, 1976). This protein, however, is absent in rats (Sata *et al.*, 1976). Except for the 74000 proline-rich apolipoprotein, all other apolipoproteins are below 26000 (Scanu *et al.*,

Fig. 1. SDS/polyacrylamide-gel electrophoresis of human and rat blood proteins

Linear-gradient gels (7.5–15%) were reduced with 2-mercaptoethanol. (a) Human serum: lane 1, men; lane 2, women. (b) Plasma from Sprague-Dawley rats: lane 1, male castrated at 2 weeks of age; lane 2, intact male; lane 3, intact female; lane 4, female castrated at 2 weeks of age.

Fig. 2. SDS/polyacrylamide-gel electrophoresis of rat plasma minus albumin fractions

Single-density (12.5%) gels were run under reducing conditions. Lane 1, castrated male; lane 2, normal male; lane 3, castrated female; lane 4, normal female.

1975). The differences observed, therefore, are probably not attributable to apolipoproteins.

The initial comparisons of serum from men and women are not as well defined as those in the rat.

Fig. 3. SDS/polyacrylamide-gel electrophoresis of Wistar rat plasma minus albumin fractions, without mercaptoethanol reduction

Linear gradient gels (5–15%): lanes 1, 2 and 3, intact males; lanes 4, 5 and 6, intact females.

This is probably due, in part, to interindividual variation, which is less of a problem with uniform strains of rats. Nonetheless, some preliminary observations may be made. No obvious differences are seen between serum of men and women in gels run under reducing conditions. For samples run without mercaptoethanol reduction (Figs. 4 and 5), two bands of approx. 92000 and 88000 appear to be variable in concentration in men, although relatively constant in women (Fig. 4). In addition, a band of 70000 appears to be present in higher concentration in men (Figs. 4 and 5).

The relative concentrations of the 92000 and 88000 mol.wt. bands in Fig. 4 were determined by computerized densitometry, to assess the relative variability. These data are presented in Table 1, and represent the integrated density of all points within the boundaries of each band. It is seen in Table 1 that the 92000 band in men is present in greater concentration than in women with a greater range in values. The average concentrations of the 88000 band are approx. equal for both sexes, but the concentrations vary over a smaller range for women than for men.

We have excluded the possibility that the observed differences may be attributed to artifacts of the albumin removal. We have shown (Mahany *et al.*, 1980) that non-albumin proteins are absent in the albumin fractions, in the 74000–78000 range of rats or the 90000 range of humans.

In summary, the present experiments demonstrate clearly the presence of sex-related differences in the patterns of circulating protein of rats and humans. These differences only become apparent once serum albumin is removed from the samples,

Fig. 4. SDS/polyacrylamide-gel electrophoresis of human serum minus albumin

Samples were not reduced with mercaptoethanol. Linear gradient (10–15%): lanes 1–9, men; lanes 10–17, women.

Fig. 5. SDS/polyacrylamide-gel electrophoresis of human serum minus albumin. Linear gradient (5–12%). Conditions are as described in Fig. 4. Lanes 1–9, men; lanes 10–17, women.

Table 1. Relative concentrations of the 92000 and 88000 molecular weight bands as determined by computerized densitometry

Integrated density is the sum of the gray levels of all the pixels within the boundaries of each band; means $\pm$ S.D. are shown.

Band (group)	Integrated density	Range
92000M	20 727 $\pm$ 8692	13 000–41 000
88000M	13 700 $\pm$ 1616	11 000–17 000
92000F	15 305 $\pm$ 2072	13 000–19 000
88000F	14 195 $\pm$ 1458	13 000–16 000

before analysis by SDS/polyacrylamide-gel electrophoresis. Although past studies have indicated differences in the levels of minor, specific proteins, between the sexes (Davidson *et al.*, 1977; Shanbhag *et al.*, 1973; Kobayashi & Takeda, 1977), the present study suggests the existence of a 'maleness' of 'femaleness' of the blood protein pattern, characterized both by the presence of different levels of individual bands and by the proportions of the bands relative to each other. Experiments are currently in progress to further characterize the nature of these patterns. It will be important in the future to determine the relationship of such patterns to the question of sex-related longevity.

This research was supported in part by NIH Grant HL 17516 and a contract from Pfizer Medical Systems. We thank C. M. Park and R. Juttila for performing the computerized densitometry.

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