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Analysis of an Unidentified Plasma Protein in Spontaneously Hypertensive Rats (SHR)

(hypertension/stroke/plasma protein)

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A band of plasma protein which was seemingly specific in SHR was detected by polyacrylamide gel electrophoresis. This protein was identified as one of the normal serum proteins in the rat, and was shown to be increased even in young stroke-prone SHR (SHRSP), especially in those with stroke-lesions as determined by single radial immunodiffusion. No alteration of this band was observed in rats with renal infarction hypertension 3 weeks after operation. This band corresponded to α_1-globulin fraction, and the characteristic increment of this protein fraction may be utilized as a marker of SHRSP, although its pathophysiological role is not yet clarified.

In previous reports, an unidentified protein was detected by gel electrophoresis in the post albumin region of the plasma from spontaneously hypertensive rat (SHR) and stroke-prone SHR (SHRSP). Although it has been reported that this protein may be related to the degree of vascular fibrosis, granulation, diffuse fibrosis, basophilic degeneration and thrombi in the heart and kidney or related to severe hypertension induced by sodium loading (1) (2), pathogenic relations between this protein and hypertension or hypertensive vascular lesions are still uncertain.

In the present study, this protein was examined by polyacrylamide gel electrophoresis in SHRSP at various ages and renal infarction hypertensive rats to analyze its relationship to hypertension and also to characterize its property by immunological methods.

MATERIALS AND METHODS

The pooled plasma of male SHRSP, Wistar-Kyoto rats (WKY), Wistar-CLEA, Donryu rats at various ages, and also 2 months old WKY with renal infarction hypertension 3 weeks after Loomis' operation (3) were used for the identification and characterization of plasma protein, and seven male SHRSP and WKY at the age of 40 days were used for the relative quantitative analysis, using immunological methods.

Blood was collected in heparinized hematocrit tubes by cutting tail arteries, except in the case of newborn rats when the blood was collected by decapitation. SHRSP and WKY, at the age of 40 days were anesthetized with sodium

pentobarbital, and the blood was collected from postcaval veins into heparinized syringes. The plasma was separated by centrifugation (3000 rpm, 15 min, 4°C).

Polyacrylamide gel electrophoresis was performed for the identification of plasma proteins by a slab gel system (4) (5). The plasma sample was diluted eleven times with 20% sucrose, and 5 μl of this sample was applied to the electrophoresis.

The protein was stained with 1% amide black 10B in 7% acetic acid solution and destained with a solution of 7% acetic acid. The relative densities of the different bands were scanned.

The characteristics of this protein were analyzed by the double diffusion technique (6) and single radial immunodiffusion (7). The antiserum of this protein was prepared by Dr. K. Moriwaki, National Genetic Institute, Mishima, Japan.

RESULTS

In the case of SHRSP with symptoms of stroke, a band of plasma protein close to the albumin was detected specifically by polyacrylamide gel

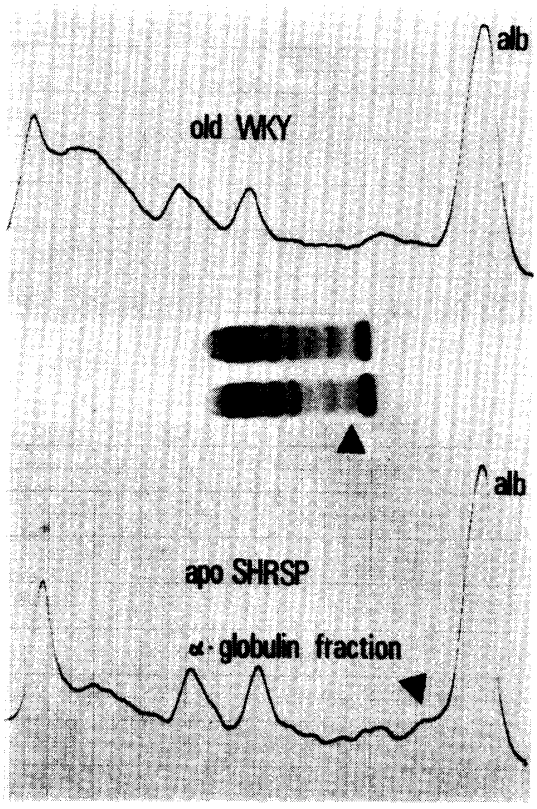

Fig. 1. Comparison of the patterns of plasma protein in SHRSP and WKY by polyacrylamide gel electrophoresis (7% polyacrylamide, 15mA, 4 hours). apo-SHRSP : SHRSP with symptoms of stroke. old WKY : the age matched normotensive Wistar-Kyoto rat.

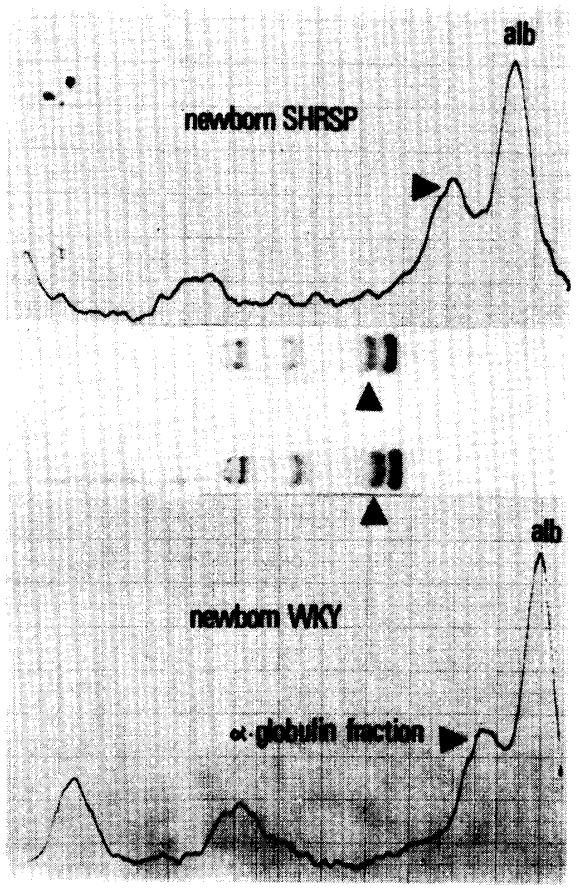

Fig. 2. Comparison of the patterns of plasma protein in SHRSP and WKY by polyacrylamide gel electrophoresis (newborn, 7% polyacrylamide, 15 mA, 4 hours).

electrophoresis but such was not evident in the adult and old WKY (Fig. 1). However, in the newborn, α_1-globulin fraction was so intensely stained both in SHRSP and WKY that no differences in plasma protein fractions could be detected (Fig. 2).

Gel electrophoresis was used to detect the band in SHRSP and WKY at the age of 40 days, blood pressures of which were 134±3 and 122 ± 4mmHg, respectively (Table I). A slightly stained band close to albumin was detected in only SHRSP (Fig. 3).

The alteration of plasma protein similar to that in SHRSP was not found

TABLE I. *Blood Pressure (BP) and Body Weight (BW) in SHRSP and WKY at Age 40 Days*

	BP (mmHg)	BW (g)
SHRSP (5)	134±3*	92±6
WKY (5)	122±4	99±1

Blood pressure was measured by a tail pulse pick-up method.
* : significant difference from WKY ($p<0.05$)
( ) : number of rats

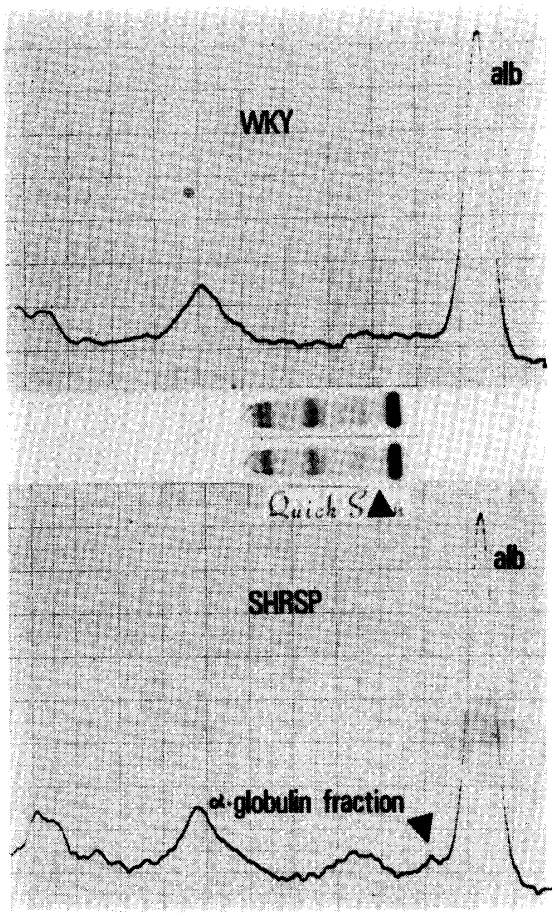

Fig. 3. Comparison of the patterns of plasma protein in SHRSP and WKY by polyacrylamide gel electrophoresis (5 weeks old, 7% polyacrylamide, 15 mA, 4 hours).

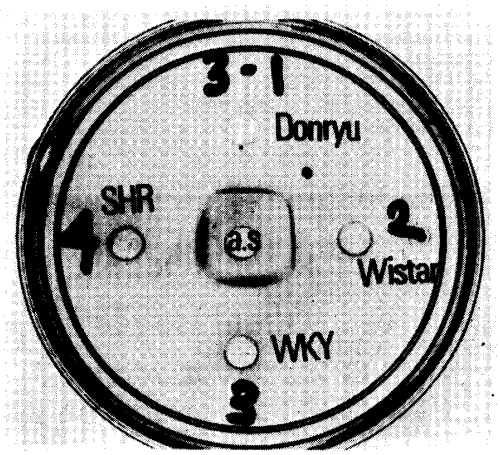

Fig. 4. Double diffusion technique showing reactions between the antiserum of the unidentified protein fraction in plasma from SHR and various other strains.

in renal hypertensive rats, the mean blood pressure of which was 171 ± 4 mmHg.

This protein was then analyzed by the double diffusion technique. The reaction was found in SHR, WKY, Wistar-CLEA and Donryu at the age of 6 months (Fig. 4). These findings indicate that this protein is one of the normal serum protein fractions in the rat.

In 40-day-old SHRSP and WKY, the relative quantity of the band of the plasma protein was estimated by single radial immunodiffusion (Fig. 5) : the

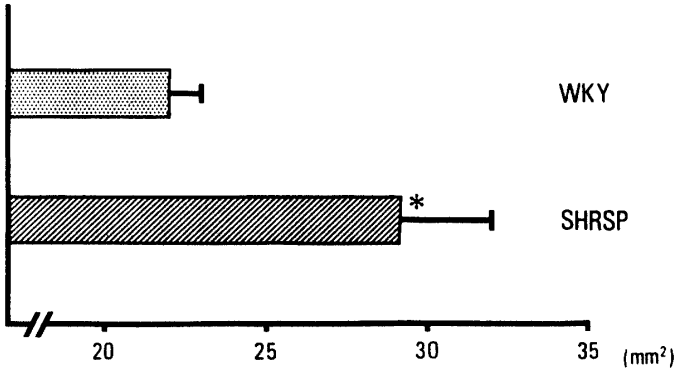

Fig. 5. Diffusion area by single radial immunodiffusion in WKY and SHRSP at age 40 days. Mean of 7 rats±standard error.
* : significant difference from WKY ($p<0.05$).

diffusion areas were $29.2 \pm 2.8\ mm^2$, and $22.0 \pm 1.0\ mm^2$ in SHRSP and WKY, respectively, and here there was a significant difference ($p<0.05$).

DISCUSSION

The present study indicated that the seemingly specific band of the plasma protein in SHRSP detected by gel electrophoresis was one of the normal serum protein fractions in the rat. However, its quantity was relatively increased in SHRSP, even at the early hypertensive stage so that it was clearly detected by gel electrophoresis in SHRSP, and no alteration was found even in WKY with renal hypertension. Therefore, the appearance of this band on gel electrophoresis depends on the quantitative difference in the plasma level especially in SHRSP.

Many serum protein fractions have been indentified in humans, but they have not been analyzed in detail in the rat. The examined fraction of the plasma from SHRSP corresponds to α_1-globulin fraction, in which α_1-trypsin inhibitor and α_1-acid glycoprotein are major proteins in the case of human plasma. A_1-trypsin inhibitor is reported to be an inhibitor of trypsin, and the synthesis of this protein is controlled by a genetic locus (8). On the other hand, α_1-acid glycoprotein binds with propranolol and chlorpromazine etc. (9). These proteins are acute phase protein, and the plasma level is increased in inflammation, malignant tumors and injury (10) (11). And also other proteins can be found in this fraction ; they are easily precipitable

glycoprotein, tryptophan poor α_1-glycoprotein, α_1-antichymotrypsin, thyroxin binding globulin, group specific component and Zn α_2-glycoprotein. It is quite reasonable that these proteins are increased in SHRSP with vascular lesions because of various pathological changes related to arterionecrosis, thrombosis or smooth muscle proliferation. Why this protein fraction is increased even in young SHRSP without vascular lesions remains to be determined. Since vascular protein synthesis is accelerated even in young SHRSP, the early increase in this fraction may be related to the proliferation of vascular wall components.

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