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Radial Immunodiffusion Assay for Rat α_1-Acid Glycoprotein

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ARNOLD, F. J. AND L. R. MEYERSON. *Radial immunodiffusion assay for rat α_1-acid glycoprotein.* PHARMACOL BIOCHEM BEHAV **37**(3) 485–491, 1990.—α_1-Acid glycoprotein (AGP) is an "acute phase protein" whose expression is altered in several human pathologies. Using antiserum from New Zealand white rabbits, a radial immunodiffusion assay for measuring AGP levels in rat plasma was developed operating in the range of 50–2500 μg/ml with high specificity. Standard curves were constructed (precipitin ring diameter^2 vs. μg/ml AGP) yielding highly linear plots ($r = .98$). The plasma concentration of AGP in spontaneously hypertensive (SHR) rats was double that of the normotensive Kyoto-Wistar (WKY) rats (208 ± 10 vs. 118 ± 5 μg/ml). AGP induction by turpentine resulted in a 14- and 26-fold increase in AGP levels in SHR and WKY rats, respectively. Induction of AGP by dexamethasone injection was examined in the SHR and WKY rat strains resulting in a 5- and 12-fold increase in AGP levels, respectively. AGP concentration in whole brain of rats was determined to be 12.7 ± 1.8 μg/g. AGP concentrations in SHR and WKY liver were also determined to be 159 ± 3 and 148 ± 5 μg/g liver tissue.

α_1-Acid glycoprotein, AGP Immunoassay Endogenous serotonin uptake modulator Biological marker Stress response

α_1-ACID glycoprotein (AGP; "acute phase protein") is produced mainly in the liver and its secretion is augmented in response to inflammation caused by physical injury, bacterial or protozoan infection or by neoplasia (17,26). AGP has been purified to homogeneity from human plasma (2) as well as other animal sources and has also been characterized molecularly in human and rat (9, 17, 28). AGP is implicated in immune function by suppressing lymphocyte responsiveness (6,7) and its production can be increased by monocyte conditioned medium (8). The synthesis of AGP can be induced by either turpentine or steroids (3, 15, 24, 25). Recently, AGP has been shown to be an allosteric modulator which stimulates serotonin uptake into human platelets by competing for the site labeled by [^{3}H]-imipramine (19).

Physically, the molecular mass of AGP is approximately 45 kD of which only 23 kD belongs to the core polypeptide. The remaining mass represents various carbohydrate moieties attached to asparagine residues, usually 3–5 depending on the species, near the N-terminus (17). These carbohydrate side chains are necessary for biological activity (2). This glycoprotein shares homology with serum retinol-binding proteins in its three-dimensional structure (23) and as such may determine its ability to bind lipophilic compounds. AGP and asialo-AGP also bind various tertiary and quaternary ammonium drugs with high affinity (29). Psychotropic drugs and catecholamines bind to human AGP with modest affinity (13,27).

Although the exact physiological role of AGP still remains unclear, several interesting biological observations have been reported. For example, plasma AGP increases in an age-related fashion (1) and appears to vary in a diurnal pattern (20,30). Additionally, patients with major depression (DSM-III) show significant increases in plasma AGP (21). In rats, it was found that a direct correlation between hepatic AGP mRNA and serum AGP levels could be made (10). In spontaneously hypertensive rats (SHR) a marked increase in number of platelets and platelet serotonin (5-HT) concentration is observed compared to the normotensive Kyoto-Wistar rat (WKY) (12). Subsequently, SHR rats were shown to have twice the amount of AGP mRNA and platelet 5-HT than the WKY rats (22).

A major difficulty in characterizing the biological aspects of AGP in laboratory animals is the lack of methodology to measure its concentration in plasma and tissues. A procedure for measuring human AGP is available (Behring Diagnostics, Inc.). However, it does not cross-react with AGP from other species.

Thus, the development of a convenient and rapid assay for rat AGP can allow for more direct physiological and pharmacological experimentation. The present report details the development of a radial immunodiffusion assay capable of measuring AGP levels in rat with high specificity, precision and accuracy.

METHOD

Animals

Three-month-old New Zealand white rabbits (Taconic Farms, Tarrytown, NY), 2 male and 2 female, weighing approximately

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2 kg were housed separately given food and water ad lib. The animals were maintained on a 12-hour light/dark cycle. After an acclimation period, rabbits were immunized and blood collected by ear vein bleeding. Male Sprague-Dawley (SD) rats (Taconic Farms) approximately 300–400 g were group housed given food and water ad lib maintained on a 12-hour light/dark cycle. Animals were sacrificed prior to use by decapitation. Male SHR and WKY rats (Taconic Farms) approximately 300–400 g were housed separately given food and water ad lib maintained on a 12-hour light/dark cycle and bled via tail cut. Blood pressures were measured with a Harvard blood pressure monitor equipped with a tail cuff. Rats were briefly etherized then bled in the initial determination of plasma AGP levels. AGP was induced in both rat strains by a subcutaneous injection (0.5 ml/100 g) of turpentine (Grumbacher Inc., New York). Following a 30-hour induction period, rats were restrained in a Plexiglas Harvard restraining tube and bled. Dexamethasone induction of AGP in SHR and WKY rats was performed by IP injection (2 mg/kg) of Decadron phosphate (Merck, Sharp & Dohme). Following a 30-hour induction period, animals were tail bled.

Materials

Purified AGP's from dog, baboon, bovine and human were obtained from Sigma Chemical Co. (St. Louis, MO) and rat AGP was obtained from Zivic-Miller Labs (Zelienopole, PA). Freund's complete adjuvant was obtained from Behring Werke AG (Marburg, West Germany). Double immunodiffusion plates were purchased from ICN Immunobiologicals (Lisle, IL). Empty immunodiffusion plates were purchased from Calbiochem-Behring (San Francisco, CA). All other reagents and chemicals were of the highest purity commercially available.

Generation of Antibodies

Rabbits of opposite sex pairs received injections with a 50/50 emulsion of antigen/Freund's complete adjuvant corresponding to antigen concentrations of 0.5 mg/ml and 1.0 mg/ml, respectively. Three 0.5 ml immunizations over the first 30 days were performed, followed by 1 booster injection at 3 months. Blood samples were collected from ear vein bleeding into heparinized tubes and centrifuged at $1000 \times g$ for 10 min. The plasma supernatant was stored at −80°C. One hundred μl aliquots of the prepared plasma were taken and the immunoreacitivity determined by double immunodiffusion.

AGP and Antibody Purity

The purity of all components in the study was analyzed by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and high performance liquid chromatography (HPLC). Protein samples were electrophoresed according to the method of Laemmli (16) on 10% polyacrylamide gels and stained with Coomassie blue R-250. Individual protein bands on the gel were scanned with a densitometer (Bio-Rad, Richmond, CA). Chromatographic analysis was performed on a Waters Associates (Milford, MA) modular HPLC system equipped with a Model U6K injector, Model 480 Lambda Max variable wavelength absorption detector, Model 6000A solvent delivery system, Model 680 system controller and Model 730 data module. Separations were performed on a DuPont G-250 column (9.4 mm × 25 cm) with an inline filter (2 micron frit). Elution of samples was achieved with a 50 mM sodium phosphate pH 7.0 containing 200 mM NaCl isocratic buffer system operating at a flow rate of 0.5 ml/min reaching a column pressure of 650–700 psi. Typically, 50–100 μg of protein was injected in a volume of 50–100 μl.

Radial Immunodiffusion Assay

Plates consisted of 1% agarose in 7 mM Na_2HPO_4, 2 mM NaH_2PO_4, 145 mM NaCl, 5 mM EDTA, pH 7.0 with 0.1% sodium azide as a preservative (4, 5, 11, 14, 18). The gel contained 20% v/v of the antiserum. Plates were prepared by combining equal volumes of diluted antiserum and 2% agarose gel solution. Temperature of the antiserum solution was maintained at 55°C just prior to preparation. The 2% agarose gel was heated at 100°C until solution was clear and then allowed to cool to 65–70°C before mixing with antiserum solution. The solutions were combined slowly to avoid production of air bubbles and slowly swirled to insure complete mixing. The combined solution was then poured slowly onto radial immunodiffusion plates and cooled at room temperature until solidified and then stored at 4°C until needed. Each plate contained 10 ml of antibody-containing agarose gel with 12 wells each accommodating 5–10 μl of sample volume. Authentic AGP ranging in concentration from 150–1250 μg/ml was used as standard controls. Plates were allowed to stand for 48 hr at room temperature to yield a fully developed precipitin ring. Subsequently, the rings were magnified by projection and quantified.

Rat Plasma Preparation

Whole blood was obtained from animals by tail bleeding and collected in microtainer plasma separator tubes (Becton Dickenson, Rutherford, NJ) containing lithium heparin and centrifuged in a Beckman microfuge for 1 min. The resultant plasma preparation was directly applied to immunodiffusion plates.

CHAPS Solubilization of Rat Liver

Whole liver from WKY and SHR rats were dissected from the animals after decapitation. The livers were rinsed briefly with water and frozen at −80°C until use. Tissue from individual rats was weighed, homogenized by a polytron and solubilized with 1.5 vol. of 50 mM Tris buffer, pH 7.5 containing 10 mM CHAPS for one hr at 25°C. The tissue preparation was then centrifuged at $200,000 \times g$ for 2 hr at 4°C in quick-seal tubes whereby 10 ml of the soluble detergent layer was removed by a syringe. Protein concentrations of both homogenate and CHAPS solubilized preparation were determined by Bio-Rad protein assay (Bio-Rad). A 10 μl sample of each liver extract was applied to previously prepared radial immunodiffusion plates in duplicate and AGP concentrations were determined.

CHAPS Solubilization of Rat Brain

Whole brain from SD rats was dissected after decapitation rinsed briefly with water. Tissue from individual rats was weighed, homogenized by a polytron and solubilized with 5 vol. of 50 mM Tris buffer, pH 7.5 containing 10 mM CHAPS for one hr at 25°C. The tissue preparation was then centrifuged at $200,000 \times g$ for 2 hr at 4°C in quick-seal tubes and 8 ml of the soluble detergent layer was removed with by a syringe. Protein concentrations of both homogenate and CHAPS solubilized preparation were determined by Bio-Rad protein assay. The CHAPS extract was then concentrated 50 times using a speed vac concentrator. A 10 μl sample of each concentrated brain extract was applied to previously prepared radial immunodiffusion plates in duplicate and AGP concentrations were determined.

Data Acquisition

Diameter of the precipitin rings was quantified to the nearest

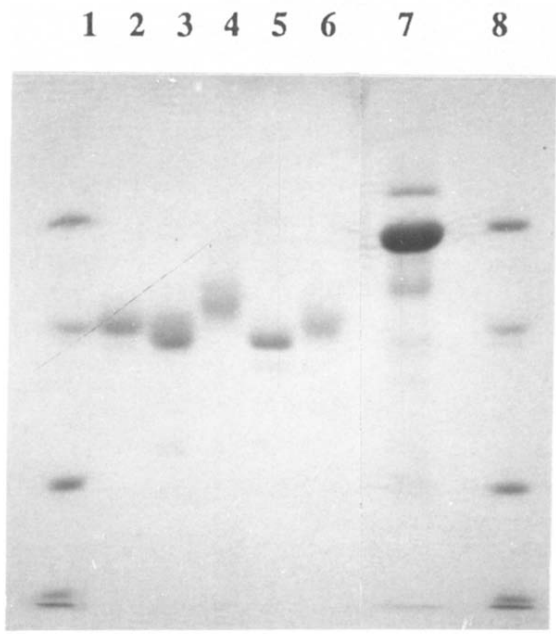

FIG. 1. SDS-PAGE analysis of AGP from various species and rabbit antisera. Lanes 1 and 8 correspond to molecular markers 66, 45, 34 and 23 kD, respectively. Lane 2 contains rat AGP; lane 3 dog AGP; lane 4 baboon AGP; lane 5 bovine AGP; lane 6 human AGP; lane 7 rabbit antiserum to rat AGP. SDS-PAGE was performed as described in the Method section where each lane contains 20 μg of protein stained by Coomassie blue. Densitometric analysis was performed to confirm visual analysis.

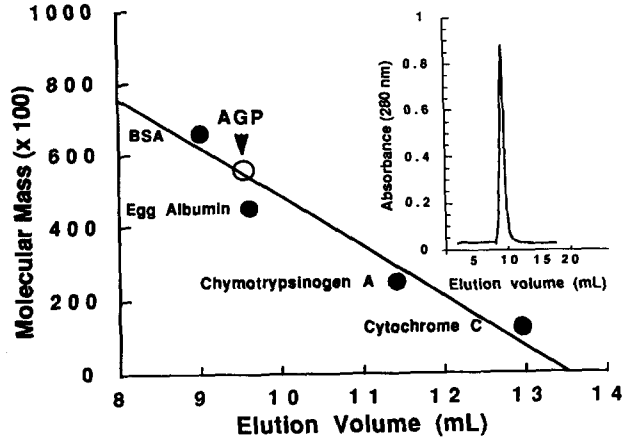

FIG. 2. Calibration curve of a Dupont GF-250 column using standard proteins is shown as molecular mass vs. elution volume. This plot was analyzed by linear regression giving a correlation coefficient of .96. AGP from different species and the antisera eluted in volumes that were all very similar. The inset chromatogram shows the homogeneity of rat AGP as separated by molecular mass. Void volume of the column was determined by the elution of ferritin.

0.1 mm by measuring a 10× amplified image of the plate using an overhead projector. Standard curves were constructed using known concentrations of AGP and plotted against their respective precipitin ring diameter ^{2} and the data was subjected to linear regression analysis.

RESULTS

Antibody/Antigen Integrity

Double immunodiffusion plates indicated that all rabbits immunized with antigen produced antibodies with significant immunoreactivity (data not shown). SDS-PAGE (Fig. 1, lane 7) and HPLC analysis of the antiserum indicates that the major molecular mass component of the antiserum corresponds approximately to 60 kD.

Purity and integrity of the various AGP's was examined by SDS-PAGE and HPLC. The electrophoretic pattern of AGP from different species (Fig. 1, lanes 2–6) showed a slight variation in the molecular characteristics of the protein. Molecular weights of AGP seem to vary slightly from species to species. AGP from rat, dog, baboon, bovine, and human gave molecular masses of 50, 48, 52, 48, and 50 kD respectively, thus indicating that these proteins are different. Densitometric analysis of the bands in Fig. 1 and HPLC analysis confirm that the AGP samples were homogeneous (Fig. 2, inset).

Assay Development

In order to ascertain the proper antiserum concentration in the agarose gel mixture, authentic rat AGP was utilized at fixed concentrations ranging from 1 μg/ml to 1000 μg/ml while the amount of antibody titer was varied. Two ml of antiserum titer was needed to attain strong precipitin rings in a final 1% agarose gel volume of 10 ml. Faint rings were visible at 500 μl of antiserum titer, However, quantification of these rings was difficult.

During the preparation of the gel mixture, it was critical that the temperature of the 2% gel solution be within the range of 60–70°C. Exceeding this temperature resulted in antibody inactivation and precipitation. Lower gel temperatures produced nonhomogeneous solidification of the media.

Assay Standardization

Initially, AGP concentrations ranging from 5–5000 μg/ml were tested. AGP concentrations <50 μg/ml produced a vague precipitin ring which was not quantifiable. AGP concentrations >2500 μg/ml gave highly irregular and nebulous rings which typically diffused out of the gel matrix and were not quantifiable. Therefore, optimal operating range for this assay exists between 50 and 2500 μg/ml. Routinely, AGP concentrations of 1250, 625, 310 and 150 μg/ml were employed as standards (Fig. 3a). The amount of error associated with each determination corresponds to 1–2%. Reproducibility between different assay plates was very consistent among similar antiserum titers. To remove variability between assay determinations, titers from different rabbits were pooled. Curves relating precipitin ring diameter^2 and AGP concentration in sample well is shown in Fig. 4. Direct linear regression of the data points gave a high correlation coefficient of .98. Standard curves using diameter ^{2} were generally employed.

Immunoreactivity of the Immunodiffusion Plates

In order to analyze the specificity and cross reactivity of the rabbit antiserum to rat AGP several different proteins as well as canine, baboon and bovine AGP were examined. Proteins were tested in a concentration range of 500–5000 μg/ml. In all cases (with the exception of rat AGP) no immunoprecipitation was observed (Fig. 3b). Similar results were also obtained with BSA, human serum albumin, human hemopexin, human α_2-macroglobulin, and egg albumin.

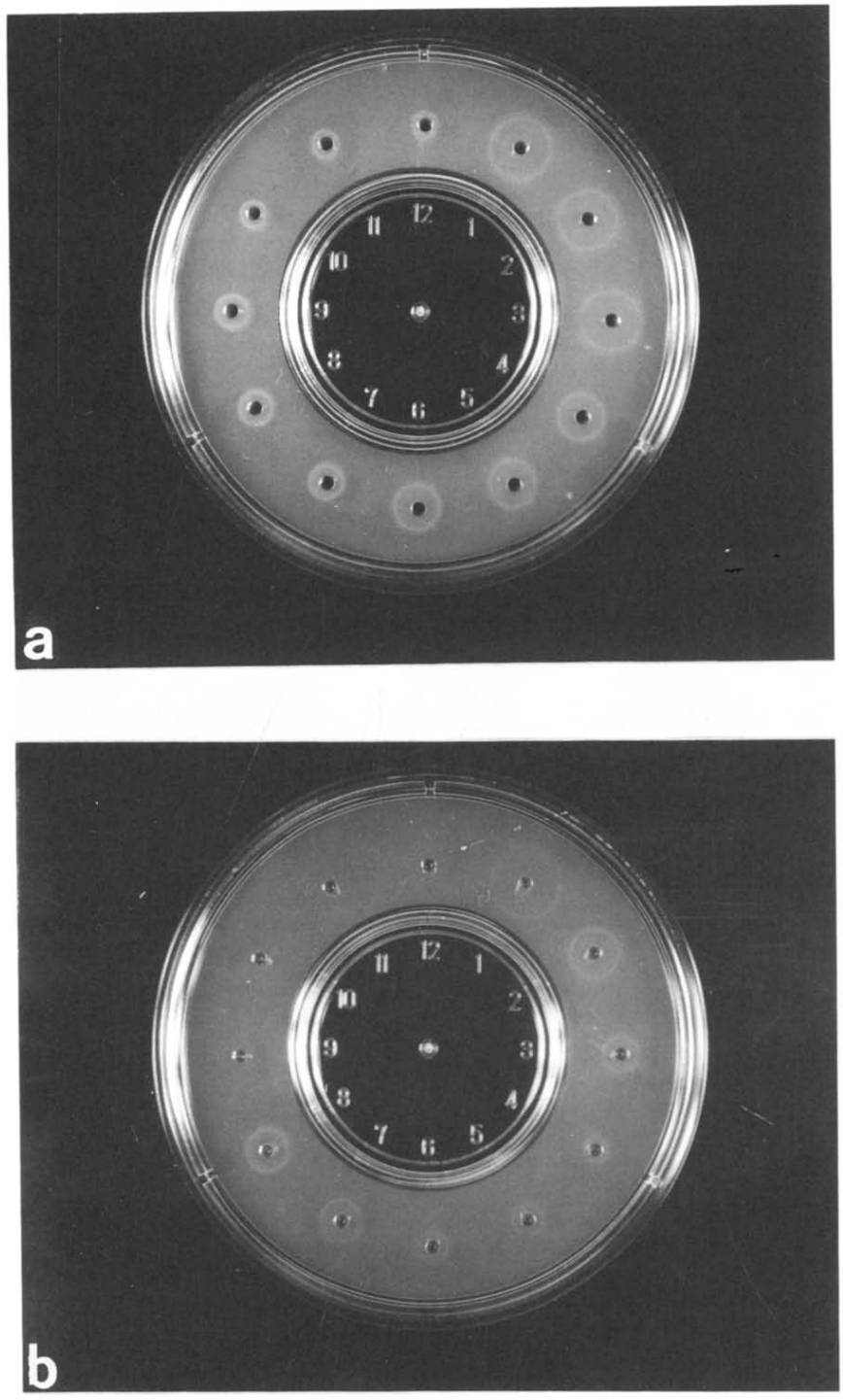

FIG. 3. Twelve-well immunodiffusion assay plate was prepared as described in the Method section containing 1% agarose gel in phosphate buffer along with antiserum. Wells were formed at time of use with gel punch. Samples were applied to wells and allowed to develop for 24 hours. The resultant rings were amplified 10× by overhead projection to insure better accuracy in measurement. In (A) wells 1, 2, 3 contain a 5 μl each sample of 1250 μg/ml authentic rat AGP. Wells 4, 5, 6 contain AGP standard of 625 μg/ml, wells 7, 8, 9 contain AGP standard of 310 μg/ml, and wells 10, 11, 12 contain AGP standard of 150 μg/ml. In (B) wells 1–4 contain a 5 μl sample of rat AGP standards of 1170, 585, 292, and 146 μg/ml respectively. Wells 5 and 6 contains 10 μl of a plasma sample from a typical WKY rat. Wells 7 and 8 contain 10 μl of a plasma sample from a typical SHR rat. Wells 9–12 contain a 5 μl sample of AGP from human, baboon, bovine, and dog respectively at a concentration of 5000 μg/ml.

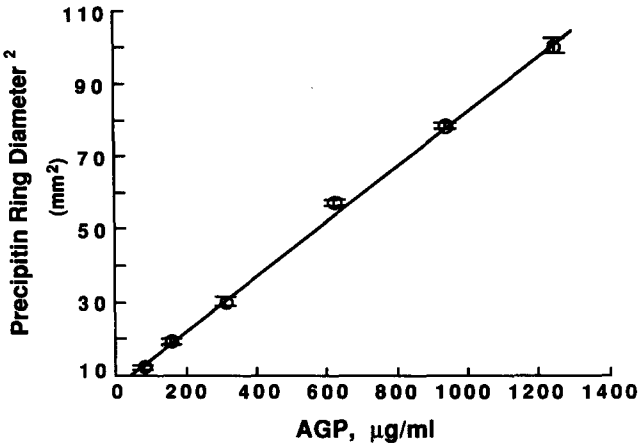

FIG. 4. Plot of rat AGP concentration vs. precipitin ring diameter^2. Data were linearly regressed using the Kaleidagraph Statistical Program (Abelbeck Inc.). The diameter of the rings were amplified 10× by projection to improve accuracy of measurement.

Application to Rat Plasma

The intended use of this assay was to measure AGP in rat plasma as well as other tissue areas. The genetic SHR model of hypertension was tested to AGP alterations because of its putative alterations in the mRNA coding for AGP (22). The Kyoto-Wistar (WKY) rat was used as a normotensive control.

The SHR animals were allowed to age until they reached hypertensive criterion at 12 weeks (diastolic pressure of 181 ± 11 mmHg). AGP concentration in SHR plasma was 208 ± 10 μg/ml, while the concentration of AGP in the plasma of normotensive WKY (diastolic pressure of 116 ± 5 mmHg) was 118 ± 5 μg/ml (Table 1). These results are statistically significant ($p<0.0001$, n = 8).

AGP induction by turpentine resulted in dramatic increases in plasma AGP levels (Table 2). In the WKY strain an increase from 105 ± 11 to 2710 ± 236 μg/ml of AGP concentration corresponding to a 26-fold increase. The SHR strain also showed a large increase from 205 ± 11 to 2829 ± 129 μg/ml of plasma AGP concentration that corresponded to a 12-fold increase. In all four test groups, n = 4. Highly significant difference was seen between the basal AGP levels ($p<0.001$), whereas no difference was seen after turpentine induction.

TABLE 1

PLASMA AGP LEVELS IN NORMOTENSIVE AND HYPERTENSIVE RATS

Strain/Age	B.P., mmHg	AGP, μg/ml
WKY/12 weeks	116 ± 5	118 ± 5
SHR/12 weeks	181 ± 11	208 ± 10

Systolic blood pressures were taken on a Harvard tail cuff measuring device (n = 8). Diasystolic measurement was difficult to obtain due to animal movement. Rats were briefly etherized before tail cutting to facilitate blood collection. Plasma samples were prepared and measured as indicated in the Method section (n = 8). Both measurements were taken 12 weeks from the animals date of birth, the time at which the SHR rats reach hypertension. Statistical analysis of these data indicates high significance in both analyses with $p<0.0001$.

TABLE 2

INDUCTION OF PLASMA AGP BY TURPENTINE AND DEXAMETHASONE

Strain	Basal AGP, μg/ml	Turpentine-Induced AGP, μg/ml	Dexamethasone-Induced AGP, μg/ml
WKY	105 ± 11	2710 ± 236	1278 ± 46
SHR	205 ± 11	2829 ± 129	1025 ± 52

These data represent quadruplicate rat plasma samples. In this case, animals were not etherized prior to blood collection, but they were restrained in a Harvard clear tube restrainer. Rats were 14 weeks of age with no alterations in blood pressures or body weight. No statistical significance was seen in the turpentine-induced AGP of the SHR and WKY rats, however, high significance was seen in the basal levels ($p<0.001$) of the SHR and WKY strains and high significance ($p<0.0001$) between basal and turpentine-induced AGP levels of both SHR and WKY strains. Dexamethasone-induced AGP levels were also showing high significance ($p<0.0001$).

Induction of AGP by dexamethasone in SHR and WKY rats resulted in a five- and twelve-fold increase in AGP plasma concentrations from (Table 2). These data reflect pre- and postdexamethasone injection AGP levels corresponding to a n = 4 with high statistical significance ($p<0.0001$) with both strains.

AGP Levels in Rat Liver

In the CHAPS solubilized protein preparations it was found that WKY and SHR livers contained 59 ± 2 and 64 ± 1 μg/ml of AGP (see Table 3) respectively ($p = 0.03$). The AGP constitutes 0.063 and 0.067% of the solubilized protein extracted from WKY and SHR liver. The concentration of AGP in liver was determined by accounting for a 2.5-fold dilution from solubilization. The efficiency of AGP extraction was previously determined to be 100% (data not shown). AGP concentrations in WKY and SHR rat livers were calculated to be 148 ± 5 and 159 ± 3 μg/g liver.

AGP Levels in Rat Brain

CHAPS solubilization of rat brains weighing 2.17 ± 0.03 g gave protein concentrations of 11.4 ± 0.5 mg/ml reflecting a 30% extraction from homogenate. In order to obtain a quantifiable signal in our assay technique, concentration of the CHAPS extract was necessary. AGP concentrations in this concentrate was 104.7 ± 14.4 μg/ml. After accounting for all concentration and dilution factors it was found that the AGP concentration in whole rat brain was 12.7 ± 1.8 μg/g. This corresponds to 0.1% of all brain protein extracted.

DISCUSSION

The role of AGP in stress, inflammation, cancer and depression remains unknown despite the extensive molecular characterization of this protein. AGP as a natural modulator and competitor of the site labeled by [^{3}H]-imipramine suggest that it may have clinical relevance in diseases associated with serotonin aberrations. Nevertheless, in order to study any of these possible conditions, a convenient method to trace the component in question is necessary. Initially, one would choose to examine the blood for any alterations in any of its components under certain biologically relevant conditions. Previously, there has been no assay available to measure rat AGP.

Investigations were targeted to assess plasma levels of AGP in

TABLE 3

RELATIVE AGP CONCENTRATIONS IN WKY AND SHR RAT LIVER

Strain	Tissue Weight (g)	Homogenate Protein (mg/ml)	Solubilized Protein (mg/ml)	AGP CHAPS (μg/ml)	AGP Liver (μg/g tissue)
WKY	18.3 ± 0.5	145 ± 8	91 ± 6	59 ± 2	148 ± 4
SHR	16.1 ± 0.2	125 ± 17	84 ± 3	64 ± 1	159 ± 3

Whole liver from both WKY and SHR rats (n = 8) were homogenized in buffer containing 10 mM CHAPS (see the Method section). Individual livers were weighed and protein concentrations determined before and after solubilization. AGP concentrations were assayed in the solubilized material and the subsequent AGP concentration in liver was determined based upon tissue weight and dilution by buffer.

the rat. In order to accomplish this task, it was decided to construct a radial immunodiffusion assay similar to one used for human studies (20,21). The use of this assay over a RIA or ELISA type assay is that 1) sample preparation is minimal, 2) preparation of the assay medium is straight forward, 3) no chemical modification of either the antibodies or the antigen is required and 4) data collection does not require any specialized equipment. The polyclonal antibodies produced for this purpose proved more than adequate in accomplishing this task. The purity of the rat AGP utilized was confirmed by the two conventional methods of HPLC and SDS-PAGE. The coomassie staining patterns of the different AGPs varied slightly around the area of 45,000 daltons. However, all of the samples analyzed showed the typical smearing that is associated with glycoproteins. The purity of the polyclonal antibodies was not a concern since the antibodies themselves were not identified. The analyses were conducted to confirm that the globular proteins were intact and not proteolysed.

The physical preparation of the agarose/antibody media was critical. For example, excessive temperatures (over 70°C) caused the antibodies to precipitate and/or become inactive. Lower temperatures (below 55°C) would cause nonhomogeneous solidification of the agarose gel media. In the assay plate, sample well was designed to accommodate a volume between 5–10 μl. Repeated application in the same well of a dilute sample would result in highly diffuse precipitin rings. Thus, samples with very low levels of AGP required concentration prior to assay. The sensitivity limits of this assay (50–2500 μg/ml) was determined by measurements of precipitin rings at specific AGP concentrations. AGP amounts <50 μg/ml did not produce a concentration dependent precipitin ring diameter or were simply not detectable. AGP concentrations >2500 μg/ml produced highly diffuse and irregular precipitin rings. Thus, the optimal operating range of the assay is between 150–1250 μg/ml AGP.

This assay is highly specific for rat AGP since precipitin rings were not detected when AGP from other species was tested. This is due to the fact that the glycosylation of the different AGP's varies from species to species (17,24) which is a major determining factor in immunorecognition. The reproducibility from assay plate to assay plate was excellent with an error <1.0%. It is recommended that internal AGP standards be included in every plate to insure accuracy.

This assay was successfully applied to measure AGP levels in rat plasma. Typically, a sample of 10 μl of undiluted rat plasma was used in the assay. The two-fold greater concentration of AGP in the SHR rats plasma compared to WKY rats correlates with our earlier observation of larger amounts of mRNA coding for AGP in SHR's (22). The AGP concentrations found in the normotensive rats are 5-fold lower than those found in normal healthy human test subjects (20).

Initially, rats were briefly etherized prior to blood collection. Ether is a known stressing agent and may influence AGP levels. AGP levels using a manual restraining device that produces less stress was compared with the ether procedure. No significant difference in AGP concentrations were seen between ether administration and manual restraint.

Induction of AGP concentrations in rat were previously observed but not measured directly (15,25). Turpentine injection induced plasma AGP concentrations in both SHR and WKY rats 30 hours postadministration. The induction of AGP in the SHR rats was lower compared to the WKY. The altered responsiveness in gene product induction may be a genetic compromise common to this rodent model of hypertension. Dexamethasone induction of AGP in SHR and WKY rats was also demonstrated. This confirms previous findings that AGP may play a role in immune regulation by acting as an endogenous antiinflamatory agent. The observations shown here prove the utility of this assay in this and perhaps a host of other biological studies that may be linked to stress and immune function.

This newly developed immunoassay can also be used for the estimation of AGP in various rat tissues. In studies where liver tissues from both WKY and SHR rats were analyzed for AGP content, it was found that the amount of AGP present was very similar, statistical significance was only marginal. These results indicate that the liver does not store this glycoprotein, for if it did we would see a difference in AGP concentrations as we observed in studies with plasma. CHAPS extractions from rat brain demonstrate that AGP can be measured effectively in whole tissue extractions. With an assay now available to assess AGP concentrations in the rat, a battery of studies can now be conducted to further elucidate AGP's role in normal and pathophysiology.

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