Aldosterone-Binding Globulin-Induced Hypertension in the Rat
A New Experimental Model


SUMMARY A thermostable urinary homologue of the plasma aldosterone-binding globulin (ABG), designated ABG-TsU, was isolated and purified by differential ultrafiltration, ion exchange chromatography, and gel filtration to electrophoretic homogeneity. Scatchard plot analysis using highly purified ABG-TsU demonstrated reversible high-affinity low-capacity binding at separate sites for aldosterone and dehydroepiandrosterone sulfate (DHEA-SO₄). ABG-TsU injected intraperitoneally (i.p.) in male rats resulted in sustained hypertension after 5 to 8 days, characterized after 12 days by no changes in plasma Na⁺ K⁺, aldosterone, or plasma renin activity (PRA). No histological changes could be detected in the kidneys, brains, or hearts, nor evidence of adrenocortical hyperplasia. This hypertension appears to be aldosterone-dependent since it is prevented by bilateral adrenalectomy or administration of a spironolactone, but not by adrenalectomy when aldosterone is given concomitantly with ABG-TsU. Hemodynamic characterization of this hypertension was carried out in rats after treatment with ABG-TsU or saline i.p. for 14 days. Cardiac output (CO) was measured using the reference sample microsphere method. ABG-TsU-treated rats had significantly higher mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), and CO, while no difference in total peripheral resistance (TPR) was detected. This new animal model of borderline essential hypertension (EH) induced by ABG-TsU, which has a reversible high-affinity binding for aldosterone, results in adrenal-dependent hypertension due to an increase in CO without any change in TPR, which remains inappropriately normal.

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KEY WORDS • experimental hypertension • aldosterone protein binding • borderline essential hypertension • hemodynamics

The role of aldosterone in the pathogenesis of essential hypertension (EH) remains controversial. Increased plasma levels of aldosterone in subjects with EH were found to be linked to a decrease in the metabolic clearance rate (MCR) of aldosterone in the face of a normal or decreased secretion rate. The decrease in the MCR of aldosterone was correlated to an increased aldosterone-binding capacity in the plasmas of these subjects, which was shown to be due to the presence of a novel specific high-affinity low-capacity aldosterone-binding globulin (ABG). Increased binding of aldosterone to ABG was shown to be elevated in subjects with EH and their blood relatives, but not in secondary hypertension or primary aldosteronism. This increase in ABG binding capacity was present in 52% of the subjects with EH; this elevation was transmitted as an autosomal dominant trait in families with a history of EH, suggesting that increased ABG binding capacity is a genetically inherited abnormality in half of subjects with EH. The presence of ABG in human and rat plasma as well as increased binding of aldosterone to ABG in EH and young spontaneously hypertensive (SHR) rats has recently been reported by other investigators.

In women taking estrogen-containing oral contraceptives, ABG-bound aldosterone was significantly elevated compared to that in control subjects and corre-
lated positively with mean arterial pressure (MAP) in the oral contraceptive-treated group only. These data indirectly point to a role for ABG in blood pressure (BP) regulation. To test this hypothesis directly, we isolated from normal human urine an apparent homologue of ABG for administration to rats. Our present study was carried out to further characterize this urinary homologue of ABG and the hypertension it induces in rats in order to further explore the possibility that ABG may be of etiological importance to at least half of the population with EH.

Methods

Pooled human urine from hospital personnel was collected during the daytime to which sodium azide was added in a concentration of at least 0.1%. After overnight refrigeration at 4°C, the urine was filtered through fine glass wool, and concentrated under positive nitrogen pressure in a 2000 ml ultrafiltration cell (Amicon Canada Ltd., Oakville, Ontario, Canada) using an Amicon UM 20 diaflo membrane retaining globular molecules of molecular weights (MW) greater than 20,000. This concentrate was forced through an Amicon XM 30 membrane (retaining molecules with MW exceeding 30,000) in the same system. The filtrate obtained was exhaustively dialyzed against water, lyophylized, and weighed. This fraction, representing urinary aldosterone-binding peak was exhaustively dialyzed against water, lyophylized, and weighed. This electrophoretically homogenous material was designated ABG-TsU.

The MW of ABG-TsU was determined by gel filtration in a 1.6 × 60 cm column of Sephadex G-75 (Pharmacia, Uppsala, Sweden). A calibration curve of logarithm of MW vs elution volume of known standards (Pharmacia calibration kit) on sodium dodecyl sulfate (SDS) polyacrylamide gel (gradient 10%–30%) in glass tubes (6 mm × 10 cm) at pH 7.0 in 0.01 M phosphate buffer was also compared to that of Staphylococcus aureus V8 (MW = 27,700, Miles Laboratories, Elkhart, Indiana), this being a standard of a very similar MW.

Isoelectric focusing of ABG-TsU was carried out in 12 × 0.6 cm polyacrylamide (30%) rods having a gradient from pH 4 to pH 6 formed with Bio-Lyte 4–6 ampholytes (2%). The gels were run for 1 hour at 150 V (constant), at 300 V for the next hour, and at 500 V for the subsequent 15 hours. They were then stained in 26% isopropanol and 10% acetic acid containing 0.4% Coomassie Blue R-250 and 0.05% acetic acid containing 0.4% cupric sulfate for at least 2 hours. The gels were destained in 12% isopropanol and 7% acetic acid and stored in H2O prior to being photographed.

A series of equilibrium dialyses were carried out in 0.05 M phosphate buffer (pH 7.4) at 4°C with a constant amount of ABG-TsU and 3H-aldosterone (or 14C-DHEA-SO4) in the presence of different amounts of unlabeled aldosterone (or DHEA-SO4). Scatchard plot analysis was carried out by plotting bound/free vs bound hormone and calculating the straight line by the method of least squares. From the slope of the line, the association constant Ka was computed.

A total of 58 male Sprague-Dawley rats (Charles River Breeding Laboratories) weighing initially between 175 and 205 g were housed four or five to a cage in a room with a 12-hour light-dark cycle. Standard Purina rat chow and tap water were available ad libitum. Following a 5-day acclimatization period (including daily handling), control levels of SBP and body weight were obtained. The SBP was measured in conscious rats from the proximal part of the tail using a cuff and a pneumatic pulse transducer coupled to a programmed electrophysymomanometer (Narco Bio-Systems, Houston, Texas). Rats were prewarmed to 39°C for 20 minutes and maintained at this temperature during blood pressure measurements in a temperature-controlled restraining device (Narco-Bio Systems). At least three good readings were averaged per measurement per rat.

The rats were divided into four groups. The first group (n = 28) received one daily i.p. injection of ABG-TsU (7 μg/rat) in 0.2 ml normal saline for 12 days. The second group of rats (n = 14) received one daily i.p. injection each of 0.2 ml normal saline, acting as a control group. The third group of rats (n = 8) underwent bilateral adrenalectomy under light ether anesthesia, following which 1% NaCl solution was substituted for drinking water. These rats received 7 μg ABG-TsU/rat/day i.p. in normal saline for 32 days. From the 14th day of ABG-TsU administration, aldosterone (2 μg/rat/day) was concomitantly administered by osmotic minipump (Alzet, Palo Alto, California) implanted subcutaneously. ABG-TsU was withdrawn on Day 32 while aldosterone administration continued through to Day 36. The fourth group of rats (n = 7) received ABG-TsU (7 μg/rat/day i.p. in 0.2 ml normal saline) for 32 days while concomitantly receiving Canrenoate (Soldactone, Searle, Montreal, Quebec,
Canada) for the first 14 days (1.5 mg/rat/day) by osmotic minipump (Alzet) implanted in the peritoneum.

Individual blood samples and organs for histological examination were collected from the first two groups of rats (those receiving only ABG-TsU or normal saline i.p. for 12 days) 12–18 hours following their last injection of ABG-TsU or saline. After anesthesia was induced by sodium pentothal (50 mg/rat i.p.), blood was collected rapidly by aortic puncture while the heart was still beating, and immediately placed in plastic tubes containing EDTA (5 mM) in an ice bath (4°C), then centrifuged and the plasma stored at −20°C until used for determination of plasma renin activity (PRA). Serum from a second aliquot of blood was stored at −20°C until used for determination of electrolytes (flame photometry), aldosterone, total plasma aldosterone-binding capacity (percentage of thermostable (ABG-Ts), and thermolabile (ABG) binding), measured by the improved procedure already described. The amount of aldosterone bound was calculated from the various percentages. These plasma parameters were correlated with each other within each group.

Adrenal glands, brains, kidneys, and hearts were removed from five control rats chosen at random and 13 ABG-TsU-treated rats with the highest SBP. The organs were fixed in buffered formalin, embedded in paraffin, and 6 μm sections mounted on glass slides and stained with hematoxylin and eosin. Slides were coded so that the pathologist examining the tissues was unaware of which treatment groups they belonged to. Adrenal glands were embedded whole and serial sections cut. Six random measurements of zona glomerulosa width (taken from the inner surface of the fibrous capsule) were made with an eyepiece microscopic micrometer at ×400 from sections containing the largest cross-sectional area of adrenal medulla.

Hemodynamic studies were carried out in additional normal male rats (initial weight 225 g) which were injected i.p. with ABG-TsU (n = 7) once daily (25 μg/0.1 ml/100 g in saline) for 14 days. Control rats (n = 7) received 0.1 ml/100 g normal saline i.p. once daily. After 14 days of treatment, rats were anesthetized (Inactin BYK, 100 mg/kg i.p.) and the CO measured using the reference sample microsphere method. Radioactive microspheres (15 μm, Co-57, New England Nuclear, Boston, Massachusetts) were injected over 20 seconds into the left ventricle, cannulated via the right carotid artery. Arterial blood was sampled at a constant rate (0.9 ml/min) 5 seconds prior to microsphere injection) for 50 seconds from a femoral artery for determination of CO. The lost blood volume was replaced by blood from a donor rat simultaneously injected at the same rate into a fem-

**Figure 1.** Left: Electrophoretic mobility of ABG-TsU compared to known standards (1 = phosphorylase b; 2 = bovine serum alumin; 3 = ovalbumin; 4 = carbonic anhydrase; 5 = soybean trypsin inhibitor; 6 = α-Lactalbmin, on SDS polyacrylamide gel. Right: Isoelectric focusing of purified ABG-TsU demonstrating microheterogeneity.
oral vein. At the time CO was determined, SBP and DBP were recorded directly. MAP was taken as DBP + \( \frac{1}{3} \) pulse pressure and total peripheral resistance (TPR) calculated (MAP = CO \times TPR).

The significance of differences between the means of two groups was assessed by Student's two-tailed \( t \) test. Correlations were determined using Pearson's correlation coefficient (\( r \)). A \( p \) of 0.05 or less was considered significant.

Results

Following ultracentrifugation, ion exchange chromatography, and gel filtration, ABG-TsU was found to be electrophoretically homogeneous (Figure 1). Its mobility was virtually indistinguishable from that of Staphylococcus aureus V8 (MW = 27,700) in SDS/PAGE electrophoresis (not shown) and very close to that of carbonic anhydrase (MW = 30,000), as shown in figure 1. The MW determination of ABG-TsU by gel filtration gave a value of 27,500, with \(^3\)H-aldosterone and \(^14\)C-DHEA-SO\(_4\) binding peaks coinciding at this MW, as illustrated in figure 2. ABG-TsU displayed microheterogeneity in isoelectric focusing, with isoelectric points at 4.76 and 4.80 (figure 1). Purified ABG-TsU displayed reversible, saturable, high-affinity low-capacity binding for aldosterone (\( K_a = 3.5 \times 10^{-9} \) M) and DHEA-SO\(_4\) (\( K_a = 1.5 \times 10^{-9} \) M) determined by equilibrium dialysis (fig. 3). These two steroids appear to bind to separate binding sites, as they do not displace each other from ABG-TsU (not shown).
Sustained hypertension developed in ABG-TsU-treated rats after 5 to 8 days (fig. 4 upper panel), SBP being already significantly higher ($p < 0.01$) in treated rats ($n = 28$) than in control rats ($n = 14$) after only 4 days of treatment. After 12 days of ABG-TsU administration, the treated rats had significantly ($p < 0.001$) higher SBP compared to control rats (table 1). The third group of rats ($n = 8$), which were bilaterally adrenalectomized and treated with ABG-TsU, did not show any increase in SBP during the first 14 days of ABG-TsU treatment (fig. 4 upper panel). Their mean SBP after these 14 days was $107 \pm 3$ mm Hg. On the 14th day, rats received aldosterone continuously (2 $\mu$g/rat/day). After 11 days of concomitant ABG-TsU and aldosterone administration, during which SBP rose steadily, these adrenalectomized rats had a SBP of $157 \pm 13$ mm Hg. When ABG-TsU treatment was withdrawn while aldosterone administration was continued, SBP normalized to $116 \pm 7$ mm Hg within 2 days. The fourth group of rats ($n = 7$) was treated for 14 days with ABG-TsU and a spironolactone that prevented the rats from becoming hypertensive (fig. 4 lower panel); their SBP remained normal at $122 \pm 4$ mm Hg on the 14th day of treatment. The SBP rose to $153 \pm 5$ mm Hg 18 days after discontinuation of the spironolactone. At this time, ABG-TsU was withdrawn, and SBP declined to $120 \pm 4$ mm Hg 2 days later.

The first group of 28 ABG-TsU-treated rats had significantly higher SBP and heart weight (corrected for body weight) than the 14 saline-treated controls (table 1). No significant differences in PRA, total or free plasma aldosterone, Na$^+$, or K$^+$ existed between the two groups. In ABG-TsU-treated rats, total bound plasma aldosterone and ABG-TsU-bound A were significantly lower compared to controls. Correlations among the various plasma parameters summarized in table 1 are shown in table 2, indicating some differences in the relationships between these parameters following ABG-TsU treatment. No histological abnormalities could be detected in ABG-TsU-treated rats. There was no evidence of renal abnormalities, arteriolar hyalinization, fibrointimal hyperplasia, fibrinoid necrosis, or glomerular injury. The brain microvasculature showed no evidence of hemorrhage or perivascular hemosiderin deposition, and no hypertrophy or hyperplasia of the zona glomerulosa could be detected.

The results of the hemodynamic studies are illus-
### TABLE 1. Group Means (± so) after 12 Days of Intraperitoneal ABG-TsU or Saline Administration

<table>
<thead>
<tr>
<th></th>
<th>Control rats</th>
<th>ABG-TsU-treated rats</th>
<th>t*</th>
<th>df*</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>119.7 ± 8.6</td>
<td>151.1 ± 15.0</td>
<td>7.45</td>
<td>41</td>
<td>0.001</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>299.6 ± 19.6</td>
<td>287.9 ± 19.8</td>
<td>1.86</td>
<td>41</td>
<td>NS</td>
</tr>
<tr>
<td>Heart wt/body wt (mg/g)</td>
<td>3.14 ± 0.30</td>
<td>3.45 ± 0.17</td>
<td>4.35</td>
<td>41</td>
<td>0.001</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>10.86 ± 3.02</td>
<td>11.80 ± 2.69</td>
<td>1.04</td>
<td>41</td>
<td>NS</td>
</tr>
<tr>
<td>Total plasma aldosterone (ng/100 ml)</td>
<td>14.90 ± 10.86</td>
<td>11.14 ± 3.34</td>
<td>1.33</td>
<td>40</td>
<td>NS</td>
</tr>
<tr>
<td>Total plasma aldosterone/PRA</td>
<td>1.47 ± 1.15</td>
<td>1.06 ± 0.90</td>
<td>1.27</td>
<td>40</td>
<td>NS</td>
</tr>
<tr>
<td>Free plasma aldosterone (ng/100 ml)</td>
<td>12.72 ± 9.33</td>
<td>9.93 ± 3.15</td>
<td>1.11</td>
<td>40</td>
<td>NS</td>
</tr>
<tr>
<td>Free plasma aldosterone/PRA</td>
<td>1.17 ± 1.03</td>
<td>0.93 ± 0.75</td>
<td>0.85</td>
<td>40</td>
<td>NS</td>
</tr>
<tr>
<td>Total bound plasma aldosterone (ng/100 ml)</td>
<td>2.18 ± 1.6</td>
<td>1.2 ± 0.81</td>
<td>2.63</td>
<td>40</td>
<td>0.02</td>
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<tr>
<td>ABG bound plasma aldosterone (ng/100 ml)</td>
<td>0.83 ± 0.72</td>
<td>0.41 ± 0.41</td>
<td>2.44</td>
<td>40</td>
<td>0.02</td>
</tr>
<tr>
<td>ABG-TS bound plasma aldosterone (ng/100 ml)</td>
<td>1.35 ± 0.93</td>
<td>0.85 ± 0.59</td>
<td>2.12</td>
<td>40</td>
<td>0.05</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/liter)</td>
<td>140.2 ± 1.52</td>
<td>139.8 ± 1.87</td>
<td>0.67</td>
<td>41</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma K⁺ (mEq/liter)</td>
<td>4.01 ± 0.20</td>
<td>4.17 ± 0.3</td>
<td>1.91</td>
<td>38</td>
<td>NS</td>
</tr>
<tr>
<td>Zona glomerulosa width (μm)</td>
<td>19 ± 3</td>
<td>18 ± 3</td>
<td>0.74</td>
<td>16</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Analysis by Student’s two-tailed t test.

### TABLE 2. Correlations within Groups After 12 Days of ABG-TsU or Saline Administration

<table>
<thead>
<tr>
<th>x</th>
<th>y</th>
<th>r</th>
<th>df</th>
<th>p</th>
<th>x</th>
<th>y</th>
<th>r</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA</td>
<td>SBP</td>
<td>+0.42</td>
<td>13</td>
<td>NS</td>
<td>PRA</td>
<td>Total bound aldosterone</td>
<td>-0.10</td>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>PRA</td>
<td>Total bound aldosterone</td>
<td>-0.19</td>
<td>12</td>
<td>NS</td>
<td>PRA</td>
<td>ABG bound aldosterone</td>
<td>-0.19</td>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>Na⁺</td>
<td>SBP</td>
<td>+0.54</td>
<td>12</td>
<td>0.05</td>
<td>Na⁺</td>
<td>Total aldosterone</td>
<td>-0.57</td>
<td>12</td>
<td>0.05</td>
</tr>
<tr>
<td>Na⁺</td>
<td>Total bound aldosterone</td>
<td>-0.56</td>
<td>12</td>
<td>0.05</td>
<td>Na⁺</td>
<td>ABG bound aldosterone</td>
<td>-0.56</td>
<td>12</td>
<td>0.05</td>
</tr>
<tr>
<td>Na⁺</td>
<td>ABG bound aldosterone</td>
<td>-0.51</td>
<td>12</td>
<td>0.1 (NS)</td>
<td>Na⁺</td>
<td>ABG-TS bound aldosterone</td>
<td>-0.57</td>
<td>12</td>
<td>0.05</td>
</tr>
<tr>
<td>Na⁺</td>
<td>ABG-bound aldosterone</td>
<td>-0.37</td>
<td>12</td>
<td>NS</td>
<td>K⁺</td>
<td>ABG-bound aldosterone</td>
<td>+0.32</td>
<td>24</td>
<td>0.1 (NS)</td>
</tr>
<tr>
<td>Heart wt</td>
<td>PRA</td>
<td>-0.54</td>
<td>13</td>
<td>0.05</td>
<td>Heart wt</td>
<td>PRA</td>
<td></td>
<td></td>
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</tbody>
</table>
treated in figure 5. ABG-TsU-treated rats had significantly higher SBP (168 ± 9 vs 122 ± 18 mm Hg, p < 0.001); DBP (127 ± 8 vs 90 ± 18 mm Hg, p < 0.001); MAP (141 ± vs 101 ± 17 mm Hg, p < 0.001); and CO (104 ± 23 vs 71 ± 19 ml/min, p < 0.02) compared to control rats. No difference in TPR was detected (1.41 ± 0.3 vs 1.60 ± 0.5 mm Hg/ml/min, p = N.S.). Pulse pressure tended to be higher in ABG-TsU-treated rats (41 ± vs 32 ± 9 mm Hg, p < 0.1) although not significantly so.

Two batches of ABG-TsU were used in these studies. All of the rats represented in figure 4, which received ABG-TsU, were treated with the same batch used to determine the biochemical characteristics reported in figures 1 and 2. A second batch of ABG-TsU was used for Scatchard plot analysis (figure 3) and the hemodynamic studies (figure 5). Some batches of purified ABG-TsU (not used in these studies) failed to bind aldosterone (after incubation with 3H-aldosterone, ABG-TsU did not co-elute with any 3H-aldosterone from a Sephadex G-75 column) and when pooled and administered to normal rats did not elevate BP, even when very high doses were used (10 control rats received 0.1 ml saline/100 g body weight i.p. once daily for 14 days after which time SBP = 124 ± 9 mm Hg, and rats similarly treated with ABG-TsU at doses of 25 µg/100 g (n = 12) and 200 µg/100 g (n = 5) had a SBP of 129 ± 14 and 126 ± 2 mm Hg respectively).

Discussion

Both ABG-TsU and plasma ABG are glycoproteins with the same MW and isoelectric points binding aldosterone reversibly with high affinity and low capacity. ABG-TsU also similarly binds DHEA-SO₄, as does plasma ABG (unpublished observation) at binding sites distinct from those binding aldosterone. It thus seems highly probable that ABG-TsU truly is a urinary homologue of plasma ABG.

The hypertension induced by ABG-TsU is characterized by normal levels of plasma aldosterone, PRA, K⁺, and Na⁺. This hormonal and electrolyte profile in plasma resembles that in EH more closely than in any secondary or experimental form of hypertension. This hypertension is clearly adrenal-dependent. Since adrenalectomy prevents any rise in BP following ABG-TsU administration (but not when aldosterone is also administered), as does administration of an aldosterone antagonist, it appears that this hypertension is caused by an interaction between ABG-TsU and aldosterone. That ABG-TsU binds aldosterone with high affinity supports his conclusion. Because we did not include a control series of adrenalectomized rats receiving only ABG-TsU for 36 days, we cannot be absolutely certain that the BP would not have risen spontaneously in the absence of aldosterone in the latter part of the course of ABG-TsU administration. Because ABG-TsU alone did not even slightly increase BP during the initial 14-day control period, this seems unlikely.

Spironolactone displaces aldosterone from human plasma ABG in vivo and in vitro by apparently competing with aldosterone, thereby increasing its turnover. This mechanism could partially be lowering BP in EH subjects with high levels of ABG-bound aldosterone. Spironolactone may prevent ABG-TsU from interacting with sufficient aldosterone to mediate a rise in BP by displacing aldosterone from ABG-TsU binding sites. If the interaction between aldosterone and ABG-TsU is prevented, no increase in BP will occur. The fact that only batches of ABG-TsU that bind aldosterone elevate BP, while those that lack this property do not, at extremely high doses, favors this conclusion. The reason for the lack of activity of certain batches of ABG-TsU (which retain all other biochemical characteristics) is unclear. Two groups of investigators have failed to confirm the existence of ABG or a similar aldosterone-binding protein distinct from transcortin. This may be due to inadequate or inappropriate conditions and methodology since the presence of a specific high-affinity binding protein for aldosterone in human plasma distinct from transcortin or albumin has been shown and the existence of ABG in both rat and human plasma has also been confirmed.

The biological half-life of ABG-TsU appears to be short, since BP normalized within 2 days following its withdrawal. The lower bound levels of aldosterone 12
Thus, the correlations between the various plasma parameters must be interpreted with caution. What does emerge, however, is that ABG-TsU treatment results in some modifications in the relationships among various plasma parameters, indicating subtle changes in their regulation.

Although ABG-TsU-induced hypertension is aldosterone-dependent, it clearly differs from mineralocorticoid hypertension due to lack of hypokalemia, absence of suppression of PRA, no elevation of plasma aldosterone, and no hyperplasia or hypertrophy of the zona glomerulosa, which also clearly distinguishes ABG-TsU-induced hypertension from the aldosterone-dependent hypertension caused by administration of another human urinary glycoprotein to rats. It thus seems unlikely that the renal effects of aldosterone and hence volume expansion play an important role in ABG-TsU-induced hypertension.

Increased CO and inappropriately normal TPR are characteristics of borderline EH and indicate an inability to vasodilate in the face of increased flow. ABG-TsU-induced hypertension in the rat has similar hemodynamic characteristics of increased MAP and CO with inappropriately normal TPR, which resembles borderline EH better than any secondary or experimental form of hypertension. The inappropriately normal TPR in ABG-TsU-treated rats indicates a relative vasoconstrictor causing an increased afterload, which probably contributes to the cardiac hypertrophy observed. Increased central blood volume due to increased venous tone has been shown to increase venous return and hence CO in borderline EH, even though blood volume is normal or low. Although we have not measured blood volume in our rat model, lack of suppression of PRA suggests that it is normal, and hence a similar mechanism could be involved in increasing CO in the early phase of ABG-TsU-induced hypertension. Longer duration studies are essential to determine whether ABG-TsU-induced hypertension evolves into a state of normal CO and increased TPR, as is the case with human EH.

Aldosterone has cardiovascular effects that can elevate BP both by increasing CO and TPR independent of its renal effects. Physiological levels of aldosterone have positive inotropic actions on isolated papillary muscle preparations, indicating a direct effect of aldosterone on myocardial contractility. Within 30 minutes to 3 hours following the administration of corticosteroids (including aldosterone) to normal human subjects, CO increases, this effect being related more to the mineralocorticoid than glucocorticoid properties of the corticosteroids, possibly by a direct effect independent of volume expansion. Application of aldosterone directly to the carotid sinus regions of rabbits or by cross circulation experiments leads to immediate increases in BP, indicating that aldosterone may have a direct resetting effect on the carotid-sinus baroreceptors by reducing their sensitivity so that a higher arterial pressure is required to produce normal baroreceptor discharge.

In an age-matched and race homogeneous group of men encompassing the entire BP spectrum, plasma aldosterone was the only component of the renin-angiotensin-aldosterone axis to correlate with MAP. Aldosterone did not change much in relation to MAP at lower BP, but increased with increasing MAP at higher BP, indicating that aldosterone is the most important component of the renin-angiotensin-aldosterone system for sustaining high BP and may be of importance for the development and maintenance of EH. Half of the subjects with EH have both increased plasma aldosterone and increased vascular reactivity, these two parameters being significantly and positively correlated in this half of the EH population. It has been suggested that aldosterone plays a pathogenic role by increasing vascular reactivity. Administration of aldosterone to human subjects increases the vascular reactivity to norepinephrine, whereas spironolactone significantly decreases it. Is this mediated via specific mineralocorticoid type receptors in vascular smooth muscle? Arterial smooth muscle cells contain receptors that bind aldosterone reversibly, with high affinity and low capacity (different in binding characteristics from renal mineralocorticoid receptors) that are perhaps involved in aldosterone-induced increases in vascular reactivity, TPR, and hence hypertension. Rats that are highly resistant to DOCA-salt hypertension have arterial smooth muscle aldosterone receptors of lower affinity than controls, which may protect them from the hypertensive vascular effects of mineralocorticoids. The presence of aldosterone receptors in non-epithelial tissue requires redefinition of the term “mineralocorticoid” and may represent a new class of “hypertensinogenic” receptors. Aldosterone has direct and immediate dose-dependent contractile effects on vascular smooth muscle in vitro due to inhibition of extraneuronal uptake of norepinephrine by aldosterone, which may be the mechanism whereby aldosterone increases vascular reactivity and hence BP in EH.

The relationship of ABG-TsU to vascular aldosterone receptors is unknown but perhaps it increases the bioavailability of aldosterone at these receptors leading to increased BP. Similarly increased levels of ABG-bound aldosterone in EH may result in increased vascular aldosterone activity. Evidence exists that plasma protein-bound steroid hormones are available for transport into tissues in vivo. In mineralocorticoid hypertension, however, the increased vascular effects of aldosterone would be due to the spillover from excessive circulating levels of free aldosterone (ABG bound levels are normal), which also results in increased renal aldosterone activity (which is not the case in EH or ABG-TsU-induced hypertension).

In conclusion, a new animal model of hypertension resembling borderline EH both hormonally and hemo-
dynamically has been developed with a homologue of ABG. In the light of previous studies on ABG showing it to have an increased binding capacity in 52% of the EH population, an elevation that is transmitted as an autosomal dominant inheritance, the present study points to a possible etiological role for ABG in a subgroup of subjects with EH.

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References
Aldosterone-binding globulin-induced hypertension in the rat. A new experimental model.
M Nowaczynski, W Nowaczynski, D Mavoungou, F Lioy, G Wilkins, K Fung and W Boyko

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