Resibufogenin Corrects Hypertension in a Rat Model of Human Preeclampsia

HOP VU,* MONICA IANOSI-IRIMIE,* SVITLANA DANCHUK,* EDD RABON,† TOSHIHIKO NOGAWA,‡ YOSHIAKI KAMANO,‡ G. ROBERT PETTIT,‡ THOMAS WIESE,§ AND JULES B. PUSCHETT*,[1]

*Department of Medicine, Section of Nephrology, and †Department of Physiology, Tulane University School of Medicine, New Orleans, Louisiana 70112; ‡the Cancer Research Institute, Arizona State University, Tempe, Arizona 85287; and §the Xavier University of Louisiana College of Pharmacy, New Orleans, Louisiana 70125

The study of the pathogenesis of preeclampsia has been hampered by a relative dearth of animal models. We developed a rat model of preeclampsia in which the excretion of a circulating inhibitor of Na/K ATPase, marinobufagenin (MBG), is elevated. These animals develop hypertension, proteinuria, and intrauterine growth restriction. The administration of a congener of MBG, resibufogenin (RBG), reduces blood pressure to normal in these animals, as is the case when given to pregnant animals rendered hypertensive by the administration of MBG. Studies of Na/K ATPase inhibition by MBG and RBG reveal that these agents are equally effective as inhibitors of the enzyme. Exp Biol Med 231:215–220, 2006

Key words: preeclampsia; hypertension; volume-expansion; pregnancy

Preeclampsia affects from 3%–10% of all pregnancies (1, 2). Hypertensive disorders in pregnancy represent the second leading cause of fetal wastage and maternal morbidity and mortality (3–5). The examination of the pathogenesis of this disorder has been hampered by the fact that there is a dearth of animal models (6, 7). This is particularly true of models that investigate and reproduce *early* events in the pathogenesis of the syndrome. The very recent work of Karumanchi *et al.*, in which an inhibitor of vascular endothelial growth factor was injected into rats, results in a florid, aggressive form of a syndrome that resembles severe human preeclampsia (8). The authors developed a model of preeclampsia in the rat, which is related to the imposition of excessive volume expansion (9). This model has many of the phenotypic characteristics of human preeclampsia, including hypertension, proteinuria, and intrauterine growth restriction (IUGR). The possibility that a circulating substance with vasoactive properties is involved in the pathogenesis of preeclampsia has been suggested (10, 11).

Dahl originally pointed out the possibility that the release of a humoral factor could be involved in the pathogenesis of "salt-sensitive" hypertension (12). This thesis was further elaborated on by de Wardener (13, 14) and by Blaustein, Hamlyn and their colleagues (15–17). These investigators provided evidence for the view that circulating substances with properties and structures resembling ouabain and/or digoxin could be involved in the pathogenesis of hypertension (18, 19). According to this thesis, these circulating inhibitors of Na/K ATPase have vasoconstrictive and inotropic as well as natriuretic properties. However, because of a defect in sodium transport, patients with salt-sensitive hypertension or volume expansion–mediated hypertension are unable to excrete sodium normally. This defect prevents them from restoring the extracellular fluid (ECF) volume to normal. As a result, the persistent volume expansion leads to continued elaboration of a circulating factor(s), causing an elevation in cellular calcium, which results in vasoconstriction, with consequent hypertension (18, 19).

We have recently determined that the excretion of the steroid bufadienolide, marinobufagenin (MBG; Ref. 20), a member of a group of compounds similar to the cardenolides, ouabain and digoxin, is elevated in this rat model of preeclampsia (21). Similar to the cardenolides, the bufadienolides have the ability to inhibit Na/K ATPase (20). Furthermore, the excretion of MBG in this rat model increases *before* hypertension develops (21). In addition, MBG administered to pregnant animals results in the

These studies were supported in part by a research grant-in-aid from Dialysis Clinic, Inc. (J.B.P.) and by the Louisiana Board of Regents Millennium Trust Excellence Fund (2001–2006)-07.

[1] To whom correspondence should be addressed at 1430 Tulane Avenue, SL45, New Orleans, LA 70112. E-mail: jpusche@tulane.edu

Received July 26, 2005.
Accepted October 12, 2005.

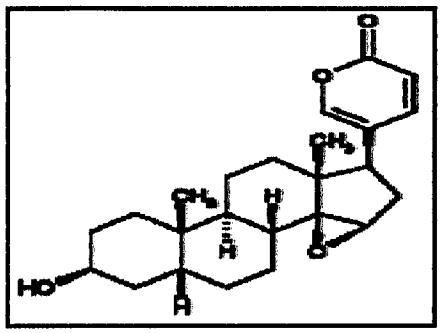
1535-3702/06/2312-0215$15.00

development of hypertension. Importantly, antibodies to MBG correct the hypertension (21, 22).

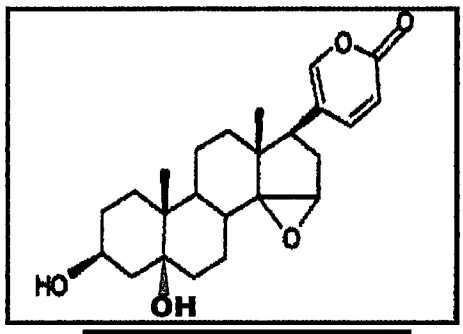
Resibufogenin (RBG) is a congener of MBG that differs from MBG only in the absence of a 5-β hydroxyl group (Fig. 1). MBG is a potent inhibitor of the α-1 isoform of Na/K ATPase (23, 24). It is a member of the bufalin family. These steroids act as cardiac inotropes and natriuretic agents, and have vasoconstrictive properties (20). We reasoned that it might be possible to antagonize the effects of MBG by administering a compound that is structurally similar to MBG but has no effect on angiogenesis. RBG is such a compound (25), although, in some *in vitro* and *in vivo* studies, it has been reported to have cardiotoxic effects (26, 27). A vasopressor effect has been reported (28). However, in the latter studies, the dose administrated was several orders of magnitude higher than that used in the current study. Based on modeling studies, we determined that RBG might be a reasonable candidate. To examine the mechanism of the antagonism of the action of MBG by RBG, we evaluated the actions of these two compounds on Na/K ATPase inhibition singly and in combination.

Materials and Methods

Animal Preparation. Female CD rats (250–300 g; Charles River, Wilmington, MA) were allowed free access to standard rat chow and tap water. They were maintained on a 12:12-hr light:dark cycle and acclimatized for 1 week before being studied. Animal care was conducted in accordance with institutional guidelines. The animals were mated with male CD rats weighing 275–300 g. Pregnancy was confirmed by the presence of vaginal plugs or by examination of vaginal smears. The pregnant females were isolated from the males. Systolic blood pressure (BP) was measured by a tail-cuff method (LifeScience Instruments, model 59; IITC Inc., Woodland Hills, CA).



Resibufogenin



Marinobufagenin

Figure 1. Structural formulas for MBG and RBG.

To determine BP, the animals were placed in a plastic restraining cage with the tail protruding. They were warmed to 37°C. BP measurements were taken sequentially until stability of the measurements was obtained. We then recorded three to five consecutive measurements. The mean value of these measurements was recorded and used. The mean (± standard error) of these values per animal were used for the periods designated in Figure 2. Measurements of BP were performed at the same time of day (the morning) on each occasion. Hypertension was considered to have been established when the animals demonstrated at least a 30 mm Hg increase in BP over their baseline values.

The animals were randomly divided into three groups. Group I: pregnant + desoxycorticosterone acetate (DOCA) + saline animals ($n = 5$) were injected initially with 12.5 mg of DOCA intraperitoneally in a depot form, followed by 6.5-mg injections of DOCA on a weekly basis. In these animals, drinking water was replaced with 0.9% saline. After hypertension was established, BP was measured daily *via* the tail-cuff method, as described in the previous paragraph. RBG was then injected in a dose of 1.53 μg/100 g animal weight daily in this group. Group II: normal pregnant animals ($n = 6$) were given daily injections of MBG in a dose of 0.765 μg/100 g animal weight. When hypertension was established, these animals were also given RBG in a daily dose of 1.53 μg/100 g body weight while the daily administration of MBG continued. BP was measured daily. Group III: control normal pregnant rats ($n = 7$) were given a daily injection of the vehicle (dimethylsulfoxide [DMSO], in which both the MBG and RBG were solubilized) only. BP was also measured in these animals.

Western Analysis. The protein concentration was determined by the Pierce bicinchoninic acid (BCA) protein assay (Woburn, MA). using bovine serum albumin as a standard. Aliquots containing 1–10 μg of membrane protein were resolved by 7% sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) run under reducing conditions, according to the method of Laemmli (29) and transferred to nitrocellulose for Western analysis. The nitrocellulose membranes were blocked with 1% instant nonfat dry milk in Tris-buffered saline, pH 7.4, containing 0.1% Triton X-100 and equilibrated with antibodies to Na/K ATPase α1 (1:2000), α2 (1:1000), or α3 (1:1000) for 1 hr at room temperature. The primary antibody was detected by anti-mouse or anti-rabbit IgG conjugated to horseradish peroxidase and visualized using the SuperSignal West Pico kit available from Pierce. Chemiluminescence was captured

on a FluorChem 8900 camera and converted to an 8-bit Tiff image. Resident software was used for spot densitometry of the Na/K ATPase. Each density measurement of the Na/K ATPase was corrected by a paired blank control of an identical spot placed immediately below the immunoreactive band.

ATPase Analysis. Na/K ATPase activity was measured in canine kidney obtained from Sigma Chemical Co. (St. Louis, MO) or rat membranes prepared by the method of Jorgensen (30). In each case, Na/K ATPase was measured using the malachite green assay of Lancetta (31). Typically, a 30–100 ng aliquot of the Na/K ATPase preparation was pre-equilibrated with inhibitor for 5 mins at 37°C and assayed in 0.1 ml of the ATPase reaction buffer containing 110 m*M* NaCl, 10 m*M* KCl, 2.6 m*M* $MgCl_2$, 1.3 m*M* ethyleneglycoltetraacetic acid, and 51 m*M* HEPES, pH 7.4. The ATPase reaction was started by the addition of 1 m*M* ATP, then incubated at 37°C for 30 mins and stopped by sequential 45-µl additions of malachite green and citrate buffer reagents. Inorganic phosphate (P_i) was measured at 570 nm using a Molecular Devices VERSAmax multiwell plate reader. The 50% inhibitory concentration (IC_{50}) for reagent inhibition was calculated from the combined data of two experiments by fitting to the equation $v = (V_{max})/(1 + 10^{[inh - logIC50]}) + nsb$. The V_{max} for the ouabain specific activity of the canine preparation is 80–97 µmol/mg/hr and typically accounts for 89%–95% of the ATPase in this preparation. The Na/K ATPase of the DOCA-treated rat preparation was 295 ± 7 µmol/mg/hr.

Statistical analyses of the *in vivo* data were performed using one-way analysis of variance (ANOVA; Fig. 2a, b) and Student's *t* test for dependent variables (Fig. 2c).

Results

The results of these studies are provided in Figure 2. Mean BP increased from 108 ± 4 to 133 ± 3 mm Hg ($P < 0.05$) in the animals that received saline plus DOCA (Fig. 2a). The superimposition of RBG reduced BP to control levels (109 ± 6 mm Hg; $P < 0.05$). In those animals rendered hypertensive by the administration of MBG, BP rose from baseline values of 108 ± 4 mm Hg to 122 ± 4 mm Hg ($P < 0.01$; Fig. 2b). The daily injection of RBG resulted in a return of the BP to normotensive levels (110 ± 3 mm Hg; $P < 0.01$). Thus, antagonism of the effects of MBG led to a return of BP to normal levels in both groups of animals. There were no changes in BP in animals administered the vehicle only (DMSO). Initial BP readings of 104 ± 5 mm Hg did not differ from those obtained after 14–21 days of gestation (104 ± 4 mm Hg; $P = 0.2$; Fig. 2c).

NA/K ATPase Isoform Expression. Samples of the Na/K ATPase from canine and rat preparations were probed with isoform-specific Na/K ATPase antibodies. Figures 3A and B provide a typical Western analysis of a 1-µg aliquot of the canine and rat preparation, respectively. The isoform expression is similar in the canine (Fig. 3A)

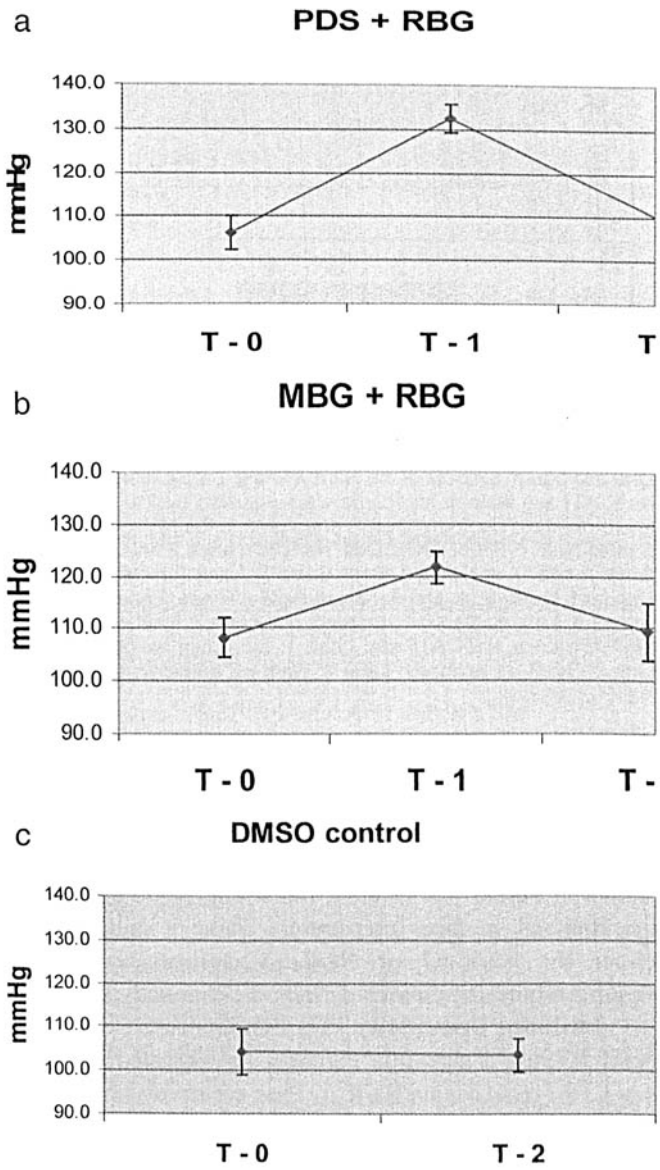


Figure 2. (a) Effects on BP (in mm Hg) of the administration of RBG to animals rendered "preeclamptic" by the replacement of their drinking water with saline and the administration of DOCA in a depot form on a weekly basis. T_0 = baseline studies; T_1 = 7–10 days of gestation; and T_2 = 7–10 days after the daily injection of RBG. Values are means ± SE. (b) Effects on BP of the daily injection of RBG in animals rendered hypertensive by the daily administration of MBG. T_0 = baseline studies; T_1 = 7–10 days of gestation, during which MBG was injected intraperitoneally daily; T_2 = mean BP after 7–10 days of daily administration of RBG while MBG injections continued. (c) DMSO control.

and rat (Fig. 3B) preparations, in which each preparation is strongly immunoreactive to the Na/K-α1 antibody but not to the Na/K-α2 and Na/K-α3 isoforms. With sample overexposure, a faint immunoreactive band to the Na/K-α3 antibody (but not the Na/K-α2 antibody [data not shown]) was visible in the canine Na/K ATPase preparation. Densitometric analysis of this area for each isoform revealed that faintly immunoreactive bands were present for both the Na/K-α2 and Na/K-α3 isoforms. The densitometric ratio of

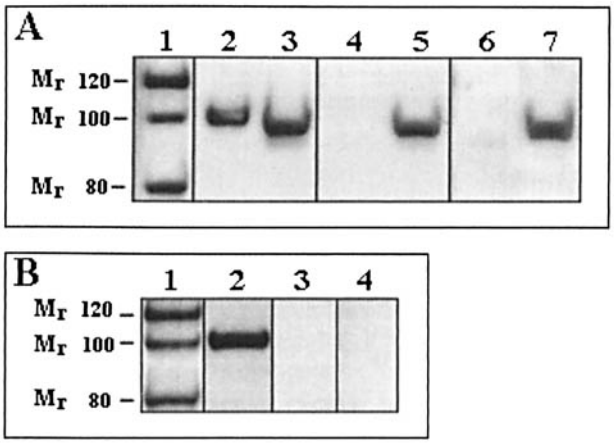


Figure 3. Isoform analysis of Na/K ATPase membrane preparation. One-microgram aliquots of the Na/K ATPase preparation or 10 μg of Na/K ATPase isoform standards were resolved by 7% SDS-PAGE and probed with isoform-specific antibodies to the Na/K ATPase. (A) Canine Na/K ATPase antibodies: Na/K-α1 (lanes 2 and 3), Na/K-α2 (lanes 4 and 5), or Na/K-α3 (lanes 6 and 7). Lane 1: molecular weight standard; Lanes 2, 4, and 6: canine Na/K ATPase; Lane 3: Na/K-α1 standard; Lane 5: Na/K-α2 standard; and Lane 7: Na/K-α3 standard. (B) Rat kidney Na/K ATPase. Lane 1: molecular weight standard; Lane 2: Na/K-α1 antibody; Lane 3: Na/K-α2 antibody; and Lane 4: Na/K-α3 antibody.

the Na/K ATPase preparation to the standard protein was 0.74, 0.07, and 0.09 for the Na/K-α1, Na/K-α2, and Na/K-α3 isoforms, respectively. The minority Na/K-α2 and Na/K-α3 isoforms were not detected in the rat Na/K ATPase shown in Figure 3B. Overall, the strong predominance of the Na/K-α1 in these preparations make it unlikely that either the Na/K-α2 or Na/K-α3 isoform contributes significantly to the measured Na/K ATPase activity.

Inhibitor Specificity. The inhibitor sensitivity of the Na/K ATPase is shown in Figure 4. As indicated in Figure 4A–C, the renal canine Na/K ATPase activity is inhibited by

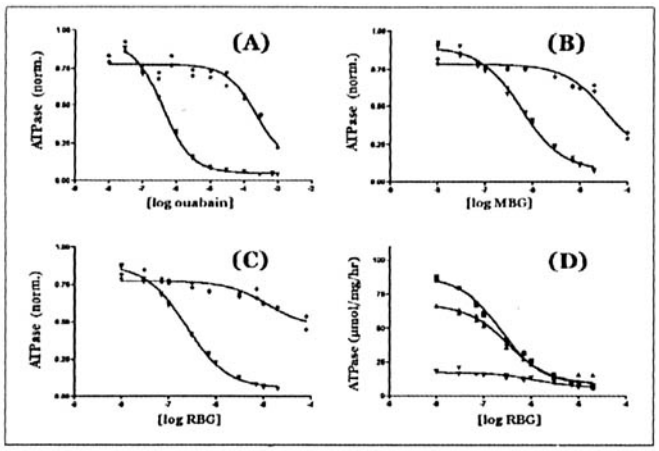


Figure 4. Inhibition of the Na/K ATPase by ouabain, MBG, and RBG. Na/K ATPase activity was measured at 37°C in the presence of the indicated inhibitor. Each tracing is a nonlinear best fit to: $v = V_{max}/(1 + 10^{[inh - \log IC50]}) + nsb$. (A–C): Canine Na/K ATPase. ◆(A–C): DOCA-treated rat membranes. (A) Ouabain inhibition. (B) MBG inhibition. (C) RBG inhibition. (D) RBG inhibition in the presence of P, 0 μ*M* MBG; >, 0.3 μ*M* MBG; and 7.0 μ*M* MBG.

ouabain, MBG, or RBG alone. The concentration dependence for each inhibitor listed in Table 1 indicates that the potency of each antagonist for the canine Na/K ATPase was similar, with an IC_{50} of 0.41×10^{-6} *M*, 0.59×10^{-6} *M*, and 0.23×10^{-6} *M* for ouabain, MBG, and RBG, respectively. This lowered apparent affinity is likely species or preparation dependent and is not attributed to the DOCA treatment because the 95% confidence interval for the IC_{50} for ouabain inhibition of the rat Na/K ATPase prepared from saline-treated control animals overlapped that of preparations prepared from the DOCA-treated rats (data not shown). Although the data in Table 1 show a lower apparent affinity for each antagonist for the rat Na/K ATPase, this preparation was also more discriminant of the antagonists, MBG and RBG. The table shows that the IC_{50} for ouabain was 215×10^{-6} *M*, whereas the IC_{50} for MBG and RBG was decreased to 33.1×10^{-6} *M* and 56.6×10^{-6} *M*, respectively. Overall, the data are consistent, in that each of the bufalins acts as an antagonist of the Na/K ATPase. Because little is known of the mechanism of inhibition by RBG, a second type of experiment was initiated to measure the Na/K ATPase activity in canine preparations pre-equilibrated at RBG concentrations sufficient to inhibit 30% or 86% of the control activity. The inhibition kinetics in the presence of both RBG and MBG could provide evidence of a mechanism dependent on homogeneous or heterogeneous effects of inhibitor binding. Figure 4D shows that the residual Na/K ATPase in the presence of RBG is inhibited by MBG at all RBG concentrations. There is a modest increase in the IC_{50} for MBG with preincubation in increasing RBG concentrations, in which the IC_{50} increases from 0.23 μ*M* (0 μ*M* RBG) to 12.4 μ*M* (7 μ*M* RBG).

Discussion

Preeclampsia most likely represents a group of diseases with similar phenotypic characteristics, rather than a single disorder (32, 33). This may explain why some preeclamptic patients deliver large babies rather than demonstrating IUGR (34). This may also be the explanation, for example, for why some patients seem to have a rather benign course, whereas others demonstrate a rapid and aggressive progression, resulting in premature birth, fetal demise, and, on occasion, unfortunately, maternal mortality. Considerable attention has been directed to the postulate that the "final common pathway" in the pathogenetic process is hypoperfusion of the maternal-fetal unit (35). In preeclampsia, the cytotrophoblastic cells fail to convert the spiral artery and its tributaries from narrow-bore, high-resistance channels into large-bore, low-resistance vessels (2). Because of the vasoconstrictive properties of MBG (20), and our discovery that its excretion is elevated *before* the development of hypertension in our animal model of preeclampsia (21), we postulated that it might have an important pathogenetic role in the generation of this syndrome. Furthermore, the administration of MBG to pregnant rats

Table 1. Antagonism of the Na/K ATPase by Ouabain-Like Steroids

	Inhibitor	IC_{50}	95% confidence interval
Canine	Ouabain	0.41×10^{-6}	$0.36–0.46 \times 10^{6}$
DOCA rat	Ouabain	215×10^{-6}	$101–456 \times 10^{6}$
Canine	MBG	0.59×10^{-6}	$0.49–0.70 \times 10^{6}$
DOCA rat	MBG	33.1×10^{-6}	$18.3–59.7 \times 10^{6}$
Canine	RBG	0.23×10^{-6}	$0.21–0.26 \times 10^{6}$
DOCA rat	RBG	56.6×10^{-6} [a]	$14.6–219 \times 10^{6}$

[a] Fit was constrained to nsb = 0.

results in hypertension, which is corrected by the injection of antibodies to MBG (21, 22).

The data from the Western blot analysis using isoform-specific antibodies indicates that both canine and rat membrane preparations are highly enriched in the Na/K ATPase α1 isoform. This is consistent with the account of Lucking *et al.*, in which measurements using quantitative polymerase chain reaction indicate that the α2 and α3 isoforms account for less than 1% of the Na/K ATPase in this preparation (36). The shape of the inhibition curve for ouabain, MBG, and RBG were consistent with the binding for ouabain, MBG, or RBG associated with the saturation of a single class of sites. Although there are species differences between canine and rat evident in the calculated IC_{50} and antagonist selectivity, the data established that MBG or RBG alone are both effective inhibitors of the Na/K ATPase. MBG inhibition of the Na/K ATPase is additive to RBG inhibition and does not restore activity to the RBG-inhibited Na/K ATPase. Thus, the mechanism of RBG-dependent recovery from MBG-associated hypertension is not easily reconciled to mechanisms involving the competitive antagonism of MBG by RBG from an inhibitory site of the Na/K ATPase or involving the interaction of the inhibitors at separate domains accessible from a common site for MBG and RBG.

In conclusion, the physiologic data are consistent with an important role for MBG in the creation of a syndrome we induced in the rat, which resembles human preeclampsia. The biochemical data seem to rule out simple explanations of the effects of MBG and RBG on the α1 isoform of the Na/K ATPase that would account for the correlation of hypertension and increased MBG noted in the rat model of preeclampsia and its physiologic reversal associated with RBG. It is not currently known whether these experimental results in the rat have relevance for the human condition. It is clear that there is a group of preeclamptic patients with a syndrome, which mimics that which we have described in our rat model (37). Accordingly, further studies to delineate the mechanism by which RBG returns the BP to normal in "preeclamptic" rats seem appropriate, and such studies are underway. Additionally, should data accrue that verify this hypothesis, Phase I and II human studies of RBG would seem to be warranted. Should they prove successful, a carefully crafted, safe clinical trial of this agent should be entertained, in our opinion. Such studies would provide the first evidence in support of a successful method for the treatment of preeclampsia short of placental and fetal delivery.

We thank Mrs. Ida Hennington for the production of this manuscript.

1. Pridjian G, Puschett JB. Preeclampsia, part I: clinical and pathophysiological considerations. Obstet Gynecol Surv 57:598–618, 2002.
2. Pridjian G, Puschett JB. Preeclampsia, part II: experimental and genetic considerations. Obstet Gynecol Surv 57:619–640, 2002.
3. Zhang J, Meikle S, Trumble A. Severe maternal morbidity associated with hypertensive disorders in pregnancy in the United States. Hypertens Pregnancy 22:203–212, 2003.
4. American College of Obstetricians and Gynecologists. Hypertension in Pregnancy. ACOG Technical Bulletin. 219:1–8, 1996.
5. Lindheimer MD. Hypertension in pregnancy. Hypertension 22:127–137, 1993.
6. Conrad KP. Animal models of pre-eclampsia: do they exist? Fetal Medicine Review 2:67–88, 1990.
7. Podjarny E, Losonczy G, Baylis C. Animal models of preeclampsia. Semin Nephrol 24:596–606, 2004.
8. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 111:649–658, 2003.
9. Ianosi-Irimie M, Vu H, Whitbred J, Pridjian C, Nadig JD, Williams MY, Wrenn DC, Pridjian G, Puschett JB. A rat model of preeclampsia. Clin Exp Hypertens 8:605–617, 2005.
10. Graves SW, Valdes R Jr, Brown BA, Knight AB, Craig HR. Endogenous digoxin-immunoreactive substance in human pregnancies. J Clin Endocrinol Metab 58:748–751, 1984.
11. Graves SW, Williams GH. An endogenous ouabain-like factor associated with hypertensive pregnant women. J Clin Endocrinol Metab 59:1070–1074, 1984.
12. Dahl LK, Knudsen KD, Iwai J. Humoral transmission of hypertension: evidence from parabiosis. Circ Res 24(Suppl):21–33, 1969.
13. de Wardener HE. The primary role of the kidney and salt intake in the aetiology of essential hypertension: part I. Clin Sci. 79:193–200, 1990.
14. de Wardener HE. The primary role of the kidney and salt intake in the aetiology of essential hypertension: part II. Clin Sci 79:289–297, 1990.
15. Blaustein MP, Hamlyn JM. Sodium transport inhibition, cell calcium, and hypertension. The natriuretic hormone/Na+–Ca2+ exchange/hypertension hypothesis. Am J Med 77(4A):45–59, 1984.
16. Hamlyn JM, Ringel R, Schaeffer J, Levinson PD, Hamilton BP, Kowarski AA, Blaustein MP. A circulating inhibitor of ($Na^+ + K^+$) ATPase associated with essential hypertension. Nature 300:650–652, 1982.

17. Hamlyn JM, Blaustein MP, Bova S, DuCharme DW, Harris DW, Mandel F, Mathews WR, Ludens JH. Identification and characterization of a ouabain-like compound from human plasma. Proc Natl Acad Sci U S A 88:6259–6263, 1991.
18. Blaustein MP. Sodium ions, calcium ions, blood pressure regulation, and hypertension: a reassessment and a hypothesis. Am J Physiol 232(5):C165–C173, 1977.
19. de Wardner HE, Clarkson EM. Concept of natriuretic hormone. Physiol Rev. 65(3):658–759, 1985.
20. Schoner W. Endogenous cardiac glycosides, a new class of steroid hormones. Eur J Biochem 269:2440–2448, 2002.
21. Vu H, Ianosi-Irimie MR, Pridjian C, Whitbred JM, Durst JM, Bagrov AY, Fedorova OV, Pridjian G, Puschett JB. The involvement of marinobufagenin in a rat model of human preelampsia. Am J Nephrol 25:520–528, 2005.
22. Durst J, Vu H, Ianosi-Irimi M, Pridjian C, Bagov A, Fedorova O, Pridjian G, Puschett JB. Antibodies to marinobufagenin reduce blood pressure in a rat model of preeclampsia. J Invest Med 52:S318, 2004.
23. Bagrov AY, Fedorova OV. Effects of two putative endogenous digitalis-like factors, marinobufagenin and ouabain, on the Na^+,K^+-pump in human mesenteric arteries. J Hypertens 16:1953–1958, 1998.
24. Fedorova OV, Kolodkin NI, Agalakova NI, Lakatta EG, Bagrov AY. Marinobufagenin, an endogenous α-1 sodium pump ligand, in hypertensive Dahl salt-sensitive rats. Hypertension 37:462–466, 2001.
25. Pettit GR, Kamano Y, Bruschweiler F, Brown P. Bufadienolides. 14. Synthesis of bufotalien, 15_α-hydroxybufalin, and resibufogmin. J Org Chem 36:3736–3739, 1971.
26. Xie JT, Dey L, Wu JA, Lowell TK, Yuan CS. Cardiac toxicity of resibufogenin: electrophysiological evidence. Acta Pharmacol Sin 4: 289–297, 2001.
27. Xie JT. Effects of resibufogenin from toad venom on isolated Purkinje fibers. Am J Chin Med, 2:187–196, 2000.
28. Iwatsuki K, Yusa T, Kataoka Y, Sato K. Experimental and clinical studies of resibufogenin. Tohoku J Exp Med 86:93–101, 1965.
29. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685, 1970.
30. Jorgensen PL. Purification and characaterization of (Na^+ plus K^+) ATPase. IV. Estimation of the purity of the molecular weight and polypeptide content per enzyme unit in preparations from the outer medulla of rat kidney. Biochem Biophys Acta 356:36–52, 1974.
31. Lancetta P, Reinach AL, Candia O. An improved assay for nanomole amounts of inorganic phosphate. Anal Biochem 100:95–97, 1997.
32. Ness RB, Roberts JM. Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications. Am J Obstet Gynecol 175:1365–1370, 1996.
33. von Dadelszen, V, Magee LA, Roberts JM. Subclassification of preeclampsia. Hypertens Pregnancy 22:143–148, 2003.
34. Xiong, X, Demianczuk NN, Buekens P, Saunders LD. Association of preeclampsia with high birth weight for gestational age. Am J Obstet Gynecol 183:148–155, 2000.
35. Granger, JP, Alexander BT, Bennett WA, Khalil RA. Pathophysiology of pregnancy-induced hypertension. Am J Hypertens 14:178S–185S, 2001.
36. Lucking K, Nielsen J M, Pedersen PA, Jorgensen PL. Na-K-ATPase isoform (alpha 3, alpha 2, alpha 1) abundance in rat kidney estimated by competitive RT-PCR and ouabain binding. Am J Physiol 271:F253–260, 1996.
37. Outland J, Alper B, Eze C, Pridjian G, Puschett JB. Human correlates of a rat model of preeclampsia. Am J Hypertens 18:185A, 2005.