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Review

Marinobufagenin, resibufogenin and preeclampsia

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ABSTRACT

The bufodienolides are cardiac glycosides which have the ability to inhibit the enzyme, Na^+/K^+ ATPase (sodium potassium adenosine triphosphatase). They are cardiac inotropes, cause vasoconstriction (and, potentially, hypertension) and are natriuretic. Evidence has accrued over time which supports the view that they are mechanistically involved in volume expansion-mediated hypertension. In this communication, the authors summarize data which support the view that the bufodienolides and, in particular, marinobufagenin (MBG) are involved in the pathogenesis of preeclampsia. In a rat model of the syndrome, MBG causes hypertension, proteinuria, intrauterine growth restriction and increased weight gain. All of these phenotypic characteristics are prevented by an antagonist to MBG, resibufogenin (RBG). The "preeclamptic" animals also develop a vascular leak syndrome, resulting in hemoconcentration. Abnormalities in the MAPK (mitogen-activated protein kinase) system play a role in the mechanism by which MBG produces the abnormalities in the pregnant rat. Studies to discover the relevance of these findings to human preeclampsia are currently underway in several laboratories and clinics.

1. Introduction

1.1. The bufodienolides

The bufodienolides are a group of steroid compounds that are classified as cardiac glycosides [1–3]. They differ from the cardenolides, separate but similar members of the cardioactive steroid group, in chemical structure (Fig. 1) and in their cellular mechanisms of action [1–3]. The bufodienolides consist of steroid nuclei with a 6-member lactone ring, while the cardenolides possess a 5-membered lactone ring [3]. The presence of some of the bufodienolides in the ancient Chinese medicines, Chan Su, Liu-Shen, Niu-Huang-Xiao-Yen and Ya-Tong-Ya [4] and their analytical cross-reaction with the major cardenolide, digoxin, has led to the interference of the bufodienolides with assays employed to determine digoxin toxicity [5,6]. The bufodienolides are active constituents in Chan Su found in the skin and venom of the toad [7], and are also present in plants [8–10]. The cardiac glycosides are so named because of their ability to act as cardiac inotropes. However, they are also capable of causing vasoconstriction, which may lead to hypertension, and, by inhibiting the ubiquitous enzyme, Na/K ATPase, of causing natriuresis [1–3].

The most widely studied of the bufodienolides is marinobufagenin (MBG). This substance has been implicated in a variety of physiological and pathophysiological circumstances. The latter include volume expansion-mediated hypertension [11–15], volume expansion and volume overload [16–19], preeclampsia [12,20–24], endothelial cell function [25,26] and cellular growth and differentiation [3,27,28].

Resibufogenin (RBG) is a bufodienolide which differs from MBG only in the absence of a hydroxyl group in the β-5 position (Fig. 1). Recent studies have demonstrated that RBG has the capability to act as an antagonist of MBG in a number of experimental circumstances. This review will focus primarily on these two compounds, discussing the role of MBG in the pathophysiology of volume expansion-mediated hypertension (including "salt-sensitive" hypertension) and in the potential therapeutic role of its antagonist, RBG.

2. Background information

2.1. Volume expansion

In the late 1950s and early in the 1960s, a topic of animated discussion in nephrology was the question as to what the physiologic mechanisms are that result in the unfailing natriuresis that follows the imposition of volume expansion [29]. It was clear that at least two factors are involved: 1) an increase in glomerular filtration rate (GFR) with an attendant increase in the filtered load of sodium and, 2) the reduction in the circulating levels of mineralocorticoids (especially

Abbreviations: MBG, marinobufagenin; RBG, resibufogenin; AT_1-AA, agonistic autoantibodies to the AT_1 receptor; MAPK, mitogen-activated protein kinase; IL-6, interleukin 6; IUGR, intrauterine growth restriction; RAS, renin–angiotensin system; Na^+/K^+ ATPase, sodium potassium adenosine triphosphatase

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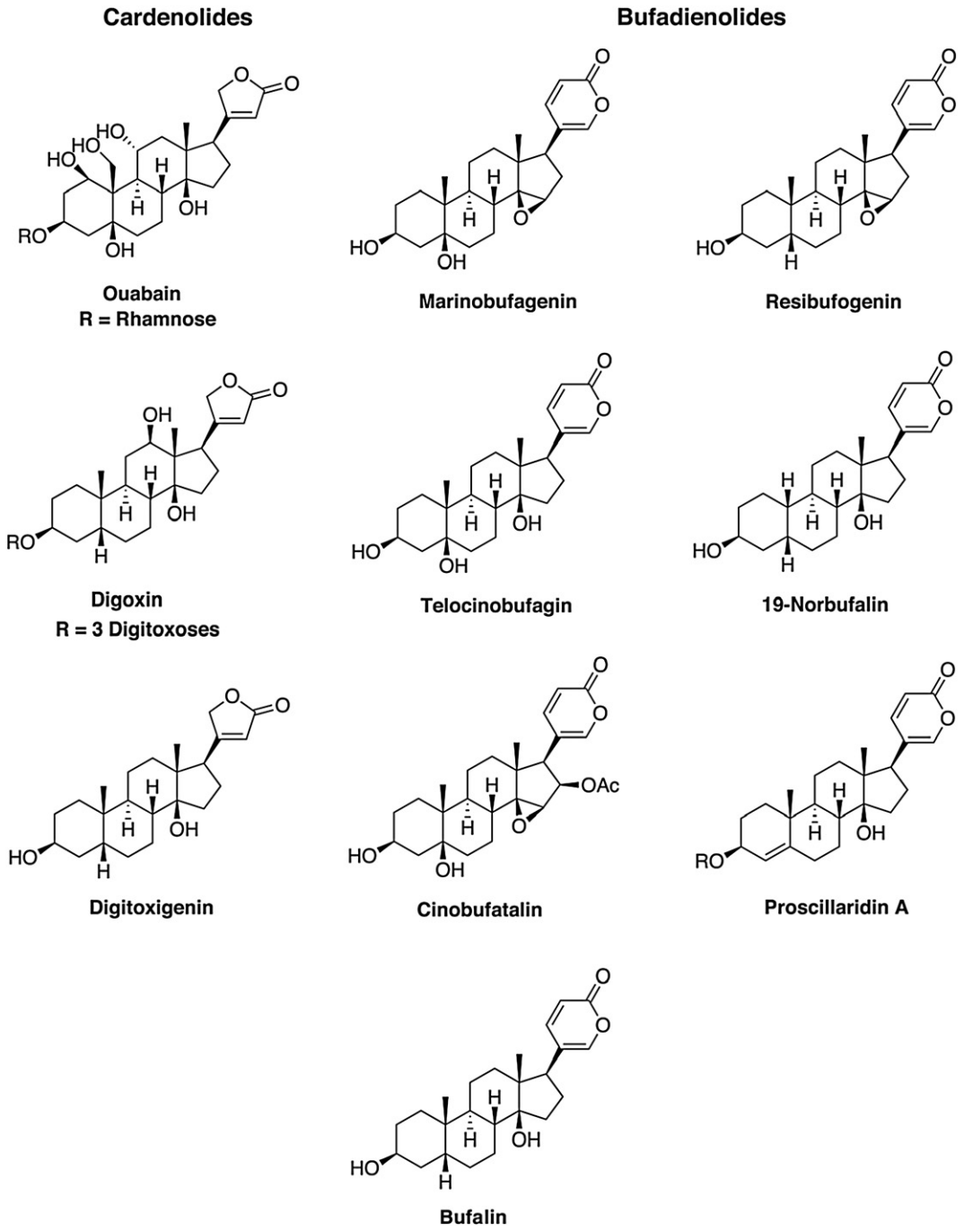

Fig. 1. Chemical structures of the bufodienolides and the cardenolides. These two groups of agents each have a steroid nucleus. However, the bufodienolides have a six-member, while the cardenolides have a five-member lactone ring. The sole difference between the chemical structures of marinobufagenin and resibufogenin is the absence of a hydroxyl group in the β-5 position of resibufogenin.

aldosterone), both playing a role. However, it soon became clear that if one corrected for these factors, preventing them from changing during acute volume expansion, natriuresis nevertheless occurred [30–32]. There arose, therefore, the concept that some other factor, so-called "third factor" must be involved [32]. Over time, the postulate that a secreted circulating hormonal factor was released as a consequence of volume expansion became the subject of intense investigation [33–37]. Accordingly, a search for such a humoral factor began. It became evident that alterations in physical factors at the renal tubular level played an important role [38–40], but could not completely explain the resultant natriuresis. In a series of ingenious experiments, De Wardener and his colleagues performed studies in which blood was cross-circulated from one dog to another, one of which had received intravenous saline [30,31]. They noted that despite careful adjustment of GFR and the filtered load of sodium in the recipient dog so that they matched or were lower than pre-expansion levels, and the provision of supraphysiologic amounts of mineralocorticoid, natriuresis nevertheless eventuated [30,31]. Subsequently, Lichardus and Pearce [35] as well as Johnston and Davis [41] confirmed these observations.

Employing toad bladder transport of sodium, Gruber, et al. showed that dialysates of plasma from volume expanded dogs interfered with the short-circuit current representing sodium transport, whereas plasma samples from control dogs had no such effect [42]. They subsequently identified a low molecular weight molecule which inhibited renal Na/K ATPase [43].This substance competed with

digoxin for two specific digoxin antibodies suggesting that it might be an endogenous digitalis-like compound [43].

3. Volume expansion-mediated hypertension

3.1. Endogenous digoxin-like factor (EDLF)

Blaustein, Hamlyn and their colleagues proposed that this so-called "natriuretic factor" interfered with sodium potassium exchange in vascular smooth muscle cells, likely due to its ability to inhibit Na/K ATPase [44]. In consequence, these cells became depolarized, resulting in an increase in intracellular sodium concentration. In turn, intracellular calcium was increased by exchange for sodium via the sodium/calcium exchanger. Consequently, the added calcium ion would become sequestered, increasing the intracellular calcium pool, resulting in vasoconstriction [45,46]. Included in the possible endogenous circulating factors, besides digoxin and, possibly, ouabain (the major cardenolide inhibitors of Na/K ATPase) is the bufodienolide, MBG. It has been demonstrated that Na/K ATPase inhibition and volume expansion are commonly observed elements in animals [11–13,15,17,18,20,47,48] and humans [19,21] in which levels of bufodienolides in blood and/or urine are elevated. In addition to MBG, these include telocinobufagin (Fig. 1) [49]. Furthermore, antibodies to MBG injected into volume expanded, hypertensive rats result in the lowering of blood pressure [20]. These agents were considered not only to directly exert vasoconstrictor effects as a result of enhanced calcium levels in vascular smooth muscle related to Na/K ATPase inhibition, but also to have direct vasoconstrictive activity unrelated to the activity of the enzyme [45,50]. Evidence for the involvement of the cardenolides, ouabain and digoxin, as the "natriuretic factor" causing hypertension has been conflicting. For details of these data, the reader is referred to a recent review [51].

3.2. Endogenous bufodienolides

As mentioned previously, the bufodienolides differ structurally from the cardenolides [1–3] (Fig. 1). In addition, they have differing effects on the isoforms of Na/K ATPase. Thus, the bufodienolides preferentially bind to the α1 isoforms, whereas the cardenolides have a predilection for the α2 and α3 forms [3]. Bufodienolides have been identified in the skin and the venom of amphibia [51,52]. MBG has also been isolated from the plasma and urine of mammalian species, including rats, dogs, and, more recently, humans [11,15,17–23,48]. The major stimulus for its secretion and elaboration appears to be volume expansion. Therefore, the concept arose that forms of hypertension related to volume expansion versus those due to vasoconstriction might involve the bufodienolides from a pathogenetic standpoint [16,53,54]. As mentioned previously, study of plasma and urine from expanded animals reveals that they most likely contain a number of cardiac glycosides, which share the capacity to inhibit Na/K ATPase.

The more recent recognition that both cardenolides and bufodienolides are likely to play a role in the effects of these cardiotonic steroids, led to better delineation as to which of these substances is responsible for their biologic effects [1–3,51].

4. Preeclampsia

Preeclampsia is a syndrome of pregnancy which occurs in 3–10% of all gestations [55,56]. It is the second leading cause of maternal and fetal morbidity and mortality in the United States and abroad. Women who receive no prenatal care are reported to be more than seven times as likely to die from complications of preeclampsia or eclampsia as women who do receive any prenatal care [57]. From 1987 until 1982, 15% of the hospitalizations for complications of pregnancy were reported to be for hypertension [58]. Preeclampsia consists in the development of hypertension and proteinuria after 20 weeks of gestation. The disorder is often complicated by the occurrence of intrauterine growth restriction (IUGR) and is associated with preterm birth. Both of the latter conditions prejudice the survival and well-being of the fetus [59]. The only current definitive therapy is delivery of the fetus and placenta. The latter appears to be a major offending organ in this disorder. There is, as yet, no completely reliable biomarker for early diagnosis [60] and no effective pharmacological therapy. Furthermore, preeclampsia is a syndrome and not a single disease process, no doubt involving multiple etiologic factors [55,56,61]. It therefore represents an example of substantial unmet medical need.

Recent research efforts in preeclampsia have produced interesting observations, which could represent novel approaches not only to its diagnosis and therapy, but also, its prevention. These include data suggestive of the importance of an imbalance between pro- and antiangiogenic factors [62,63], agonistic autoantibodies to the AT 1 receptor [64] and the possibility that circulating inhibitors of Na/K ATPase play important roles in human preeclampsia [21–23,53,65,66] and in an animal model of the syndrome [20]. Pregnancy is nature's own experiment in volume expansion. During gestation, women gain an additional 40–50% of extracellular fluid (ECF) volume [67, Fig. 2]. Given the data summarized above indicating that volume expansion is a major stimulus for MBG secretion and elaboration, the authors and their colleagues established a model of preeclampsia in the rat. The animals were volume expanded by replacing their drinking water with saline and injecting desoxycorticosterone acetate (DOCA) in a depot form on a weekly basis [68]. The result was the development of a syndrome with many of the phenotypic characteristics of human preeclampsia [69]: hypertension, proteinuria, IUGR, excessive weight gain and increased urinary excretion of MBG [14,20,68,70]. In addition, the diameters of the decidual vessels dissected from the uteri of the "preeclamptic" animals were narrower than those obtained from normal pregnant animals [14, Fig. 3]. Interestingly, their perfusion with a solution containing MBG caused a further constriction of 36%, whereas the vessels from normal pregnant animals did not respond to the bufodienolide [14]. These observations indicate "sensitization" of the arterioles to MBG in this preeclamptic model. Furthermore, the injection of antibodies to MBG resolved the hypertension. In confirmation of the involvement of MBG in the preeclamptic syndrome, Federova, et al. have reported that an antibody to MBG lowered elevated blood pressure in pregnant rats rendered hypertensive by treatment with high salt diets [12,13].

Of special interest is the fact that several aspects of the preeclamptic process can be reproduced by excessive volume expansion and the involvement of MBG. Of crucial importance in successful gestation is the process of "placentation" [71]. Shortly after implantation, a group of specialized cells, the cytotrophoblasts (CTBs),

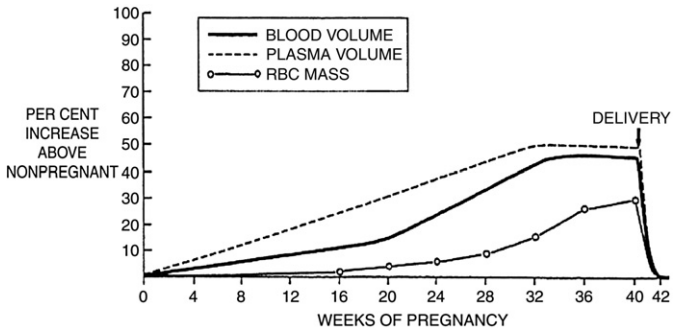

Fig. 2. Increments of blood and plasma volumes throughout pregnancy.
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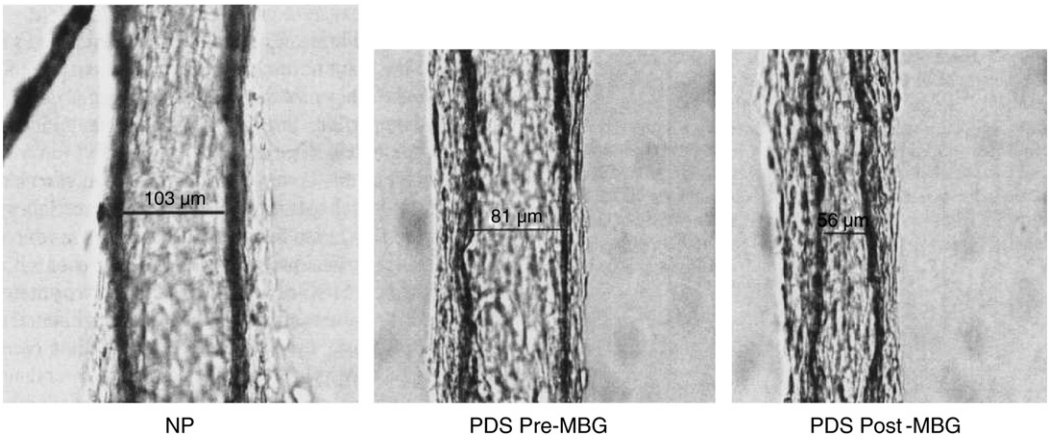

Fig. 3. Representative magnified images of the uterine arterioles dissected from normal pregnant (NP) and "preeclamptic" (PDS) rats. The initial arteriolar diameters of the PDS rats were significantly narrower (93.6 ± 3.5 µm) than those taken from the uteri of normal pregnant animals (110.6 ± 6.1 µm; $p<0.05$). The arterioles from the PDS rats constricted by an average of 36% when compared to pretreatment vessel diameter measurements. This vasoconstriction was not seen in the arterioles of the normal pregnant animals. Reproduced (with permission of the editors) from: Am J Nephrol 2005;25:520–528.

derived from the placenta, invade the myometrium [72]. Their purpose is to remodel the vasculature of the uterus such that high resistance, small bore vessels are transformed into large diameter, low resistance channels [71–73]. Presumably, this process occurs for the purpose of increasing blood flow to the maternal–fetal unit to assure proper delivery of basic nutrients and oxygen. Therefore, the effects of MBG on CTB function were examined in CTB cells in tissue culture [27,74]. It was determined that MBG-induced impairment of CTB function occurs via activation of Jnk, p38 and Src, leading to increased apoptosis and IL-6 secretion. The enhancement of the apoptotic effects of MBG on CTB could be attenuated by p38 inhibition [28].

Because the degree to which expansion of the ECF volume exceeds the increment in red cell mass, pregnant patients develop so-called "physiologic anemia" [67]. However, preeclamptic patients have been noted to have hematocrit values higher than those in normal pregnant subjects. These observations led to the concept that preeclamptic patients are hemoconcentrated. This aspect of human preeclampsia was reproduced in the rat model.

Thus, normal nonpregnant females had mean hematocrit values of 0.44 ± 0.01, while in normal, pregnant females, this value is 0.34 ± 0.02 and in "preeclamptic" rats, the mean was 0.38 ± 0.02. All three of these levels differed statistically from each other ($p<0.05$). Substantial evidence has accrued that preeclampsia represents an endothelial cell disorder [75,76]. Therefore, it has been proposed that, along with other evidence, these findings suggest a "vascular leak syndrome" with fluid transferring from the intravascular to the interstitial space [77,78, Fig. 4]. Furthermore, it has been proposed that endothelial cell dysfunction and oxidative stress are regularly observed phenomena in preeclampsia [76]. These matters were addressed in a DOCA-salt rat model of volume expansion-mediated "essential" hypertension [16] and in the rat model of preeclampsia described above [79] as well as in *in vitro* studies [25]. In the former animals, the production of superoxide anion was increased over control [16]. In the preeclamptic model, mesenteric endothelial-dependent relaxation responses and aortic nitric oxide (NO) production were significantly decreased despite increased aortic eNOS expression. Furthermore, the scavenging of reactive oxygen species or increased tetrahydrobiopterin levels normalized the relaxation responses, aortic NO production and aortic superoxides and peroxynitrile levels [80].

5. MBG and angiogenic factors

The authors endorse the concept that preeclampsia represents a syndrome with multiple etiopathogenetic factors [53,54,60,81]. That these factors may show some interrelationship in the pathogenetic process of this disorder also seems likely. In addition, the issue of the sequence of the involvement of these factors in the events leading to hypertension, proteinuria, IUGR, "vascular leak", and perhaps neurological abnormalities [82], remains to be determined. In the evaluation of the time sequence of these events, the authors have compared the time course of the appearance of abnormalities in angiogenic factors relative to the appearance of the increased secretion and elaboration of MBG [14]. In preliminary studies performed in the expansion model of preeclampsia [68] the timing of the increased excretion of MBG [14] was compared to that of changes in pro- and antiangiogenic factors [83]. Preliminary data reveal no changes in angiogenic factors at the 3–5 day gestational period in "preeclamptic" rats. At this time, MBG excretion in the urine is already elevated [14, Fig. 5]. Reductions in VEGF and increases in SFlt-1 began at the 7–10 day time-period (Agunanne, et al, unpublished observations). Furthermore, RBG, given early in pregnancy, prevented angiogenic imbalance as well as the phenotypic characteristics of the preeclamptic syndrome in this rat model [83].

6. Neurologic complications of preeclampsia

Given the vascular leak syndrome induced by MBG and its presence in our "preeclamptic" animals [79], its role was investigated in human brain monolayer endothelial cells [84]. Preliminary data indicate that, as was the case in mesenteric postcapillary venules [79], the addition of MBG to endothelial cell monolayers obtained from human brain vasculature resulted in evidence of the induction of hyperpermeability [84]. This breach of the blood/brain barrier has been confirmed in preliminary studies involving the injection of Evan's blue dye in PDS rats (Bake, et. al, unpublished observations). Given the elevation in MBG blood levels observed in human preeclamptic patients [21–23], these data could implicate MBG in the causation of neurologic symptoms and abnormalities in this hypertensive syndrome of pregnancy as previously described [82,85,86]. In experiments performed in pregnant rats, acute elevations of blood pressure resulted in autoregulatory breakthrough, blood/brain barrier disruption and cerebral edema [87]. The latter investigation also provided evidence in the acute hypertensive rat model that pregnancy may prevent hypertensive remodeling of cerebral arteries [88].

7. Conclusions and perspectives

It now seems well established that preeclampsia is not a single disease process, but a syndrome with multiple etiologic factors

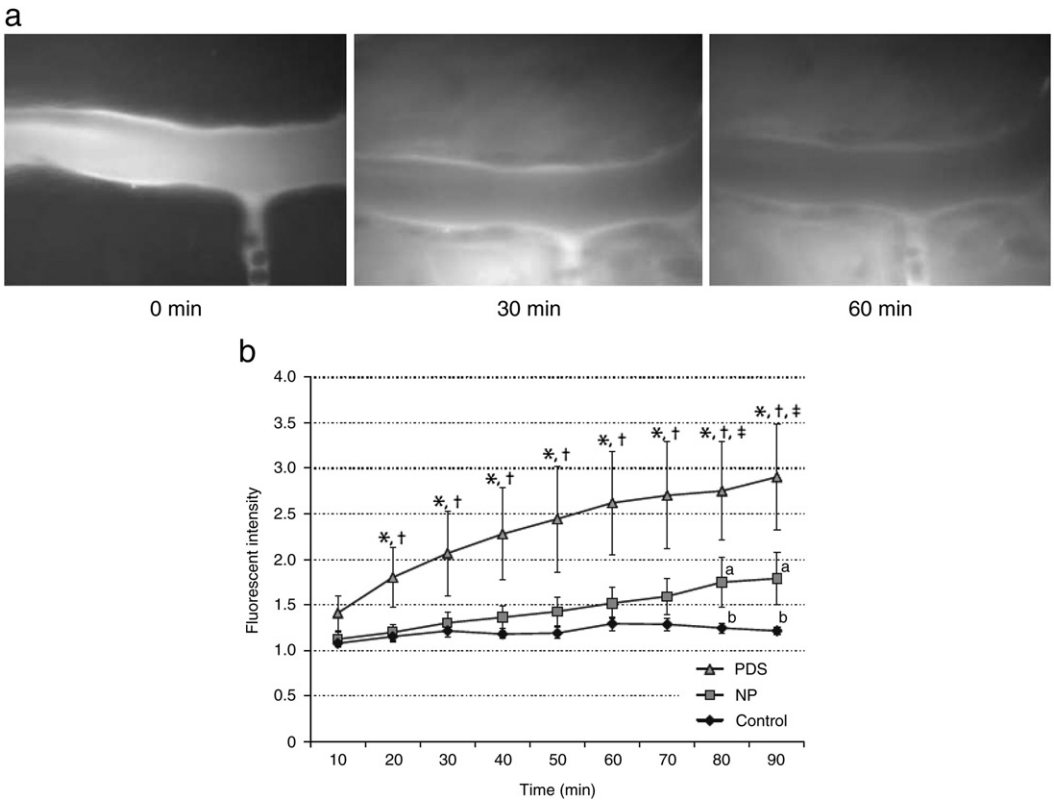

Fig. 4. a. Representative study demonstrating the effect of MBG on vascular leakage in a single rat mesentery postcapillary venule. Images shown were obtained prior to the injection (0 min) and at 30 and 60 min after the bolus injection of 200 nM MBG. FITC-albumin extravasation into the extravascular space is virtually complete by 60 min after MBG injection. b. Comparison of vascular leakage in mesenteric postcapillary venules in three groups of female rats: control = nonpregnant female animals ($n=5$); normal pregnant (NP) rats ($n=9$); pregnant animals administered DOCA and saline (PDS) ($n=9$). NP rats showed leakage of dye at 80–90 min ($p<0.05$). PDS rats showed significant leakage beginning at 20 min ($p<0.05$) when compared to control and NP rats. $^{*}p<0.05$ vs. control, $^{\dagger}p<0.05$ vs. NP, and $^{\ddagger}p<0.006$ vs. control.
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yielding similar phenotypic characteristics. Accordingly, it will not be possible to develop a single test to predict its forthcoming superventiion, but rather a panel of tests will be required. Excessive volume expansion before the 8–10 week gestation period in sensitive pregnant patients may serve as a stimulus for the elaboration of excessive amounts of the bufodienolide, MBG, (and perhaps others). This sequence of events could result in the development of a reliable predictive test. Data are also available in the animal model, but not yet in human subjects, indicating that MBG is elevated prior to both the onset of angiogenic imbalance and the development of hypertension and proteinuria. Of critical importance in these animal studies, is the fact that the early administration of the antagonist of MBG, RBG, prevents angiogenic imbalance as well as preventing the animal "preeclamptic" syndrome. Studies are currently underway in which sequential analyses of MBG and markers of pro- and antiangiogenic factors are to be examined for their appearance in human subjects with preeclampsia compared to those obtained in normal pregnant patients. Studies are also planned to evaluate polymorphisms/mutations in sodium transporters in these two patient populations to determine if those patients with elevated MBG levels are also found to have abnormalities in sodium transporters.

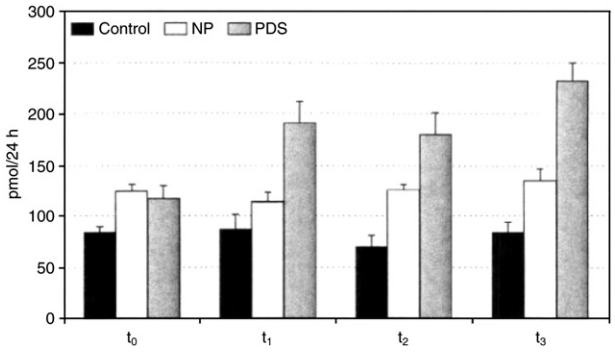

Fig. 5. Time sequence of the MBG urinary excretion pattern in control rats (C), normal pregnant animals (NP) and pregnant animals treated with DOCA weekly injections and whose drinking water had been replaced with saline, rendering them "preeclamptic" (see text) (PDS rats). Time periods: t_0 = baseline, nonpregnant state; t_1 = 3–5 days of pregnancy; t_2 = 7–10 days of pregnancy; t_3 = 16–19 days of pregnancy. MBG excretion was already statistically significantly elevated at time t_1 although the animals did not become hypertensive and proteinuric until time t_2.
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What constitutes the continued stimulus for MBG secretion once hemoconcentration has appeared, despite the fact that the patient remains expanded with substantial fluid having been extravasated into the interstitial compartment and resulting in a reduction in intravascular volume? We postulate that at this time in the gestation process, MBG has exerted its baleful effects on CTB function. Accordingly, normal vascular remodeling of the decidual vessels has been prevented and hypoperfusion of the maternal–fetal unit has occurred. The ischemic/hypoxic stimuli resulting from the restricted blood flow to these organs could cause the continued increase in MBG levels [89,90]. It is clear from animal and *in vitro* experiments, that MBG may be complicit in the "vascular leak" that typifies the preeclamptic syndrome. The challenge remains to demonstrate these observations elicited in the animal model, in human subjects. If the latter can be proven, the reward is potentially quite high: that is, that RBG may serve as an important new preventative as well as a therapeutic agent. Also of potential value and importance may be interference in the pathogenetic pathway with inhibition, for

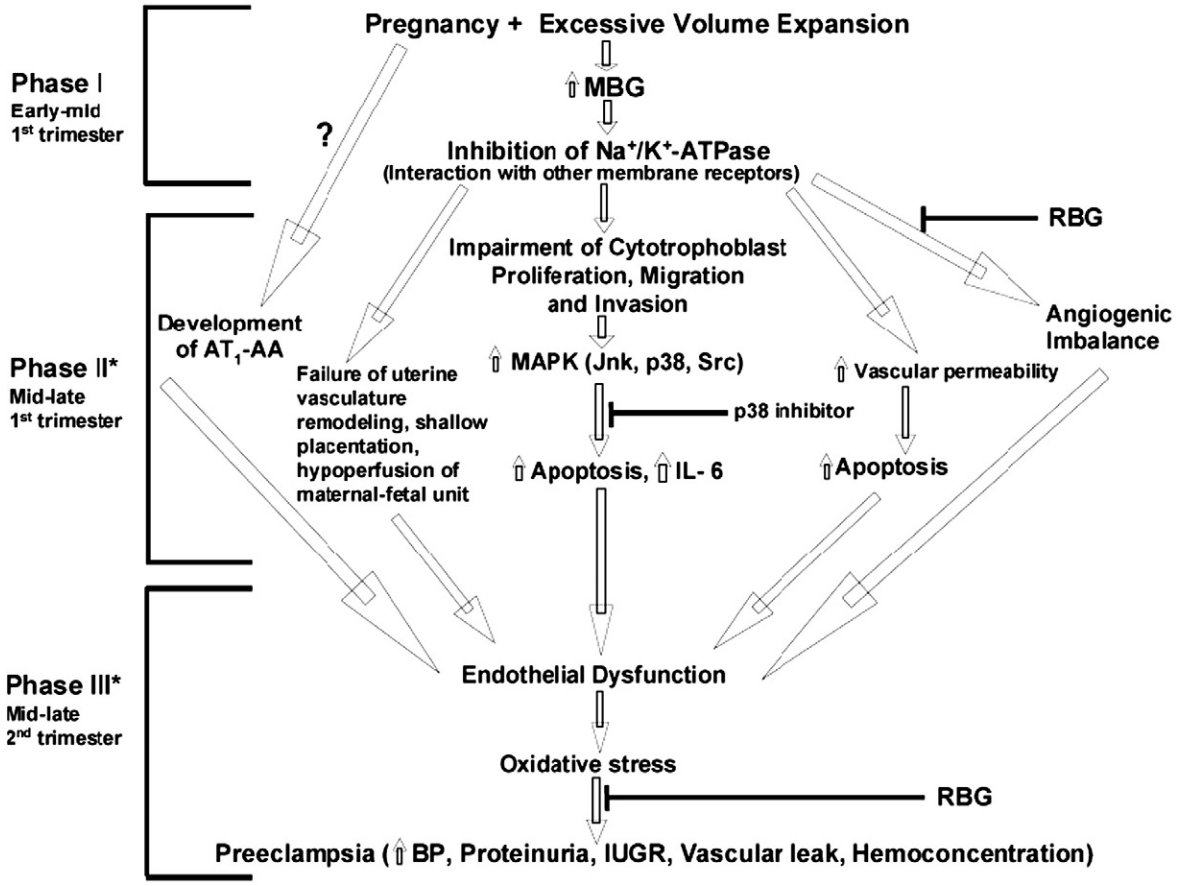

Fig. 6. Proposed three-phase model of the involvement of MBG in the pathogenesis of preeclampsia: Phase I (early-mid first trimester): initially, excessive volume expansion occurs in early pregnancy, related to difficulty in sodium disposal. The latter is postulated to be due to an inherited or acquired defect in sodium excretion. This causes elevated levels of MBG. MBG exerts its effects by inhibiting Na^+/K^+-ATPase. It may also interact with other membrane receptors. Phase II (mid-late first trimester): the elevated levels of MBG cause cytotrophoblast dysfunction and, consequently, defective placentation. This results in impaired uterine vascular remodeling and hypoperfusion of the maternal–fetal unit. The resultant hypoxia and ischemia, lead to continued elevation of MBG levels and, additionally, to an imbalance in angiogenic factors. Early administration of RBG prevents this sequence of events, including the development of angiogenic imbalance. Development of agonistic autoantibodies to the AT1 receptor may also initiate endothelial dysfunction. Additionally, MBG causes increased vascular permeability resulting in leakage from the intravascular compartment and consequent hemoconcentration. The latter further compromises blood flow to the maternal–fetal unit. The cytotrophoblast dysfunction is mediated by alterations in the MAPK system which stimulates apoptosis causing disruption of endothelial cell layers. Inhibition of the activity of p38 prevents the MBG-induced enhancement of apoptosis and IL-6 secretion. Phase III (mid-late second trimester): all of these abnormalities culminate in the production of endothelial cell dysfunction and oxidative stress. These phenomena result in the induction of the syndrome of preeclampsia including hypertension, proteinuria, IUGR, vascular leak and hemoconcentration. RBG administration early in pregnancy prevents the development of this syndrome and its phenotypic characteristics. Abbreviations: MBG = marinobufagenin, RBG = resibufogenin, RAS = renin–angiotensin system, AT1-AA = agonistic autoantibodies to the AT1 receptor, MAPK = mitogen-activated protein kinase, IL-6 = interleukin 6, BP = blood pressure, IUGR = intrauterine growth restriction. *The pathogenetic mechanisms occur at an earlier time than the appearance of the phenotypic features.

example, of p38 or cytokines such as IL-6. These entities appear to be involved in the final pathway by which excessive MBG produces its noxious effects. In Fig. 6 are provided concepts of a three-phase process by which preeclampsia occurs. It will be noted that many of the proposed events have been demonstrated thus far only in the animal model. However, based upon the evidence emanating from many laboratories and clinics, it would appear that potentially exciting observations will result, allowing for the control of preeclampsia, a disease process with often devastating consequences.

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References

[1] W. Schoner, Endogenous cardiac glycosides, a new class of steroid hormones, Eur. J. Biochem. 269 (2002) 2240–2448.
[2] W. Schoner, G. Scheiner-Bobis, Endogenous cardiac glycosides: hormones using the sodium pump as signal transducer, Semin. Nephrol. 25 (2005) 343–351.
[3] W. Schoner, G. Scheiner-Bobis, Endogenous and exogenous cardiac glycosides: their roles in hypertension, salt metabolism, and cell growth, Am. J. Physiol. Cell Physiol. 293 (2007) 509–536.
[4] K. Luojun, Z. Ke, National Commission of Chinese Pharmacopoeia, Pharmacopoeia of Peoples Republic of China, vol. 2, Chemical Industry Press, Beijing, 2005, p. 316.
[5] D.A. Biddle, P. Datta, A. Wells, A. Dasgupta, Falsely elevated serum digitoxin concentrations due to cross-reactivity of water-extractable digitoxin-like immunoreactivity of Chinese medicine Chan SU: elimination of interference by use of a chemiluminescent assay, Clin. Chim. Acta 300 (1–2) (2000) 151–158.
[6] Y. Liang, A. Liu, S. Qin, J. Sun, M. Yang, P. Li, D. Guo, Simultaneous determination and pharmacokinetics of five bufadienolides in rat plasma after oral administration of Chansu extract by SPE-HPLC method, J. Pharm. Biomed. Anal. 46 (2008) 442–448.

[7] J.R. Brubacher, D. Lachmanen, P.R. Ravikumar, R.S. Hoffman, Efficacy of digoxin specific Fab fragments (Digibind®) in the treatment of toad venom poisoning, Toxicon 37 (1999) 931–942.
[8] L. Krenn, B. Kopp, Bufadienolides from animal and plant sources, Phytochemistry 48 (1) (1998) 1–29.
[9] P.S. Steyn, F.R. van Heerden, Bufadienolides of plant and animal origin, Nat. Prod. Rep. 15 (4) (1998) 397–413.
[10] K.C. Huang, The Pharmacology of Chinese Herbs, CRC Press, Boca Raton, 1993, pp. 114–117.
[11] O.V. Fedorova, M.I. Talan, N.I. Agalakova, E.G. Lakatta, A.Y. Bagrov, Endogenous ligand of α1 sodium pump, marinobufagenin, is a novel mediator of sodium chloride-dependent hypertension, Circulation 105 (2002) 1122–1127.
[12] O.V. Fedorova, A.S. Simbirtsev, N.I. Kolodkin, A.Y. Kotov, N.I. Agalakova, V.A. Kashkin, N.I. Tapilskaya, A. Bzhelyansky, V.A. Reznik, E.V. Frolova, E.R. Nikitina, G.V. Budny, D.L. Longo, E.G. Lakatta, A.Y. Bagrov, Monoclonal antibody to an endogenous bufadienolide, marinobufagenin, reverses preeclampsia-induced Na/K-ATPase inhibition and lowers blood pressure in NaCl-sensitive hypertension, J. Hypertens. 26 (2008) 2414–2425.
[13] O.V. Fedorova, N.I. Kolodkin, N.I. Agalakova, A.R. Namikas, A. Bzhelyansky, J. St-Louis, E.G. Lakatta, A.Y. Bagrov, Antibody to marinobufagenin lowers blood pressure in pregnant rats on a high NaCl intake, J. Hypertens. 23 (4) (2005) 835–842.
[14] H. Vu, M. Ianosi-Irimie, C. Pridjian, J. Whitbred, J. Durst, A. Bagrov, O. Fedorova, G. Pridjian, J.B. Puschett, Involvement of marinobufagenin in a rat model of human preeclampsia, Am. J. Nephrol. 25 (2005) 520–528.
[15] O.V. Fedorova, N.I. Kolodkin, N.I. Agalakova, E.G. Lakatta, A.Y. Bagrov, Marinobufagenin, an endogenous α-1 sodium pump ligand, in hypertensive Dahl salt-sensitive rats, Hypertension 37 (2001) 462–466.
[16] S. Danchuk, S. Sukhanov, D. Horvat, M.N. Uddin, J.B. Puschett, Effects of resibufogenin in experimental hypertension, Am. J. Nephrol. 28 (2008) 8–13.
[17] A.Y. Bagrov, O.V. Fedorova, R.I. Dmitrieva, A.W. French, D.E. Anderson, Plasma marinobufagenin-like and ouabain-like immunoreactivity during saline volume expansion in anesthetized dogs, Cardiovasc. Res. 31 (1996) 296–305.
[18] O.V. Fedorova, E.G. Lakatta, A.Y. Bagrov, Endogenous Na,K pump ligands are differentially regulated during acute NaCl loading of Dahl rats, Circulation 102 (2000) 3009–3014.
[19] A.I. Fridman, S.A. Matveev, N.I. Agalakova, O.V. Fedorova, E.G. Lakatta, A.Y. Bagrov, Marinobufagenin, an endogenous ligand of alpha-1 sodium pump, is a marker of congestive heart failure severity, J. Hypertens. 20 (2002) 1189–1194.
[20] H. Vu, M. Ianosi-Irimie, S. Danchuk, E. Rabon, G.R. Pettit, T. Wiese, J.B. Puschett, Resibufogenin corrects hypertension in a rat model of human preeclampsia, Exp. Biol. Med. 231 (2006) 215–220.
[21] H.C. Gonick, Y. Ding, N.D. Vaziri, A.Y. Bagrov, O.V. Fedorova, Simultaneous measurement of marinobufagenin, ouabain, and hypertension-associated protein in various disease states, Clin. Exp. Hypertens. 20 (5&6) (1998) 617–627.
[22] I.V. Averina, N.I. Tapilskaya, V.A. Reznik, E.V. Frolova, O.V. Fedorova, E.G. Lakatta, A.Y. Bagrov, Endogenous Na/K-ATPase inhibitors in patients with preeclampsia, Cell Mol. Biol. 52 (2006) 19–23.
[23] D.A. Lopatin, E.K. Ailamazian, R.I. Dmitrieva, V.M. Shpen, O.V. Fedorova, P.A. Doris, A.Y. Bagrov, Circulating bufadienolide and cardenolide sodium pump inhibitors in preeclampsia, J. Hypertens. 17 (1999) 1179–1187.
[24] P.J. Hilton, R.W. White, G.A. Lord, G.V. Garner, D.B. Gordon, M.J. Hilton, L.G. Forni, W. McKinnon, F.M.D. Ismail, M. Keenan, K. Jones, W.E. Morden, An inhibitor of the sodium pump obtained from human placenta, Lancet 348 (1996) 303–305.
[25] M. Uddin, D. Horvat, E.W. Childs, J.B. Puschett, Marinobufagenin causes endothelial cell monolayer hyperpermeability by altering apoptotic signaling, Am. J. Physiol. Regul. Integr. Comp. Physiol. 296 (2009) R1726–R1734.
[26] M.N. Uddin, F. Hunter, S.S. Glaser, D. Horvat, E.W. Childs, J.B. Puschett, Marinobufagenin impairs proliferation and triggers enhanced vascular permeability in rat lung microvascular endothelial cells, J. Invest. Med. 56 (2008) 481.
[27] M.N. Uddin, D. Horvat, S.S. Glaser, S. Danchuk, B.M. Mitchell, D.E. Sullivan, C.A. Morris, J.B. Puschett, Marinobufagenin inhibits proliferation and migration of cytotrophoblast and CHO cells, Placenta 29 (2008) 266–273.
[28] M.N. Uddin, D. Horvat, S.S. Glaser, B.M. Mitchell, J.B. Puschett, Examination of the cellular mechanisms by which marinobufagenin inhibits cytotrophoblast function, J. Biol. Chem. 283 (26) (2008) 17946–17953.
[29] H.W. Smith, Salt and water volume receptors: an exercise in physiologic apologetics, Am. J. Med. 23 (4) (1957) 623–652.
[30] I.H. Mills, H.E. De Wardener, C.J. Hayter, W.F. Clapham, Studies on the afferent mechanism of the sodium chloride diuresis which follows intravenous saline in the dog, Clin. Sci. 21 (1961) 259–264.
[31] H.E. De Wardener, I.H. Mills, W.F. Clapham, C.J. Hayter, Studies on the efferent mechanism of the sodium diuresis which follows the administration of intravenous saline in the dog, Clin. Sci. 21 (1961) 249–258.
[32] F.C. Rector Jr., G.V. Giesen, F. Kiil, D.W. Seldin, Influence of expansion of extracellular volume on tubular reabsorption of sodium independent of changes in glomerular filtration rate and aldosterone activity, J. Clin. Invest. 43 (3) (1964) 341–348.
[33] J.M. Hamlyn, M.P. Blaustein, Sodium chloride, extracellular fluid volume, and blood pressure regulation, Am. J. Physiol. 251 (4 Pt 2) (1986) F563–F575.
[34] H.E. De Wardener, E.M. Clarkson, Concept of natriuretic hormone, Physiol. Rev. 65 (3) (1985) 658–759.
[35] B. Lichardus, J.W. Pearce, Evidence for a humoral natriuretic factor released by blood volume expansion, Nature 5021 (1966) 407–409.
[36] L.K. Dahl, K.D. Knudsen, J. Iwai, Humoral transmission of hypertension: evidence from parabiosis, Circ. Res. 24 (Supp. 1) (1969) 21–23.
[37] F.C. Rector Jr., M. Martinez-Maldonado, N.A. Kurtzman, J.C. Sellman, F. Oerther, D.W. Seldin, Demonstration of a hormonal inhibitor of proximal tubular reabsorption during expansion of extracellular volume with isotonic saline, J. Clin. Invest. 47 (1968) 761–773.
[38] B.M. Brenner, J.L. Troy, T.M. Daugharty, I.F. Ueki, D.P. Nicholas, C.F. Wong, On the mechanism of inhibition in fluid reabsorption by the renal proximal tubule of the volume-expanded rat, J. Clin. Invest. 50 (1971) 1596–1602.
[39] B.M. Brenner, R.W. Berliner, J.L. Troy, Relationship between extracellular volume and fluid reabsorption by the rat nephron, Am. J. Physiol. 217 (1) (1969) 6–12.
[40] B.M. Brenner, K.H. Galchuk, R.I. Keimowitz, R.W. Berliner, J.L. Troy, N. Green, The relationship between peritubular capillary protein concentration and fluid reabsorption by the renal proximal tubule, J. Clin. Invest. 48 (1969) 1519–1531.
[41] C.I. Johnston, J.O. Davis, Evidence from cross circulation studies for a humoral mechanism in the natriuresis of saline loading, Proc. Soc. Exp. Biol. Med. 121 (4) (1966) 1058–1063.
[42] V.M. Buckalew Jr., F.J. Martinez, W.E. Green, The effect of dialysates and ultrafiltrates of plasma of saline-loaded dogs on toad bladder sodium transport, J. Clin. Invest. 49 (1970) 926–935.
[43] K.A. Gruber, J.M. Whitaker, V.M. Buckalew Jr., Endogenous digitalis-like substance in plasma of volume expanded dogs, Nature 287 (1980) 743–745.
[44] M.P. Blaustein, Sodium ions, calcium ions, blood pressure regulation, and hypertension: a reassessment and a hypothesis, Am. J. Physiol. 232 (5) (1977) C165–C173.
[45] R.E. Purdy, Summary of studies of changes in vascular reactivity caused by natriuretic hormones, Clin. Exp. Hypertens. 20 (5&6) (1998) 705–716.
[46] T. Iwamoto, Vascular Na+/Ca2+ exchanger: implications for the pathogenesis and therapy of salt-dependant hypertension, Am. J. Physiol. Regul. Integr. Comp. Physiol. 290 (3) (2006) R536–R545.
[47] A.Y. Bagrov, N.I. Agalakova, V.A. Kashkin, O.V. Fedorova, Endogenous cardiotonic steroids and differential patterns of sodium pump inhibition in a NaCl-loaded salt-sensitive and normotensive rats, Am. J. Hypertens. 22 (5) (2009) 559–563.
[48] O.V. Fedorova, D.E. Anderson, E.G. Lakatta, A.Y. Bagrov, Interaction of NaCl and behavioral stress on endogenous sodium pump ligands in rats, Am. J. Physiol. Regul. Integr. Comp. Physiol. 281 (2001) R352–R358.
[49] Y. Komiyama, X.H. Dong, N. Nishimura, H. Masaki, M. Yoshika, M. Masuda, H. Takahashi, A novel endogenous digitalis, telocinobufagin, exhibits elevated plasma levels in patients with terminal renal failure, Clin. Biochem. 38 (2005) 36–45.
[50] R.G. Woolfson, J. Graves, F.S. LaBella, J.F. Templeton, L. Poston, Effect of bufalin and pregnanes on vasoreactivity of human resistance arteries, Biochem. Biophys. Res. Commun. 186 (1) (1992) 1–7.
[51] A.Y. Bagrov, J.I. Shapiro, O.V. Fedorova, Endogenous cardiotonic steroids: physiology, pharmacology, and novel therapeutic targets, Pharmacol. Rev. 61 (1) (2009) 9–38.
[52] B.T. Clarke, The natural history of amphibian skin secretions, their normal functioning and potential medical applications, Biol. Rev. 72 (1997) 365–379.
[53] J.B. Puschett, The role of excessive volume expansion in the pathogenesis of preeclampsia, Med. Hypotheses 67 (2006) 1125–1132.
[54] F.J. Haddy, Endogenous digitalis-like factor or factors, N. Engl. J. Med. 316 (1987) 621–623.
[55] G. Pridjian, J.B. Puschett, Preeclampsia, part I: clinical and pathophysiological considerations, Obstet. Gynecol. Surv. 57 (2002) 598–618.
[56] G. Pridjian, J.B. Puschett, Preeclampsia, part II: experimental and genetic considerations, Obstet. Gynecol. Surv. 57 (2002) 619–640.
[57] A.P. Mackay, C.J. Berg, H.K. Atrash, Pregnancy-related mortality from preeclampsia and eclampsia, Obstet. Gynecol. 97 (4) (2001) 533–538.
[58] C.L. Scott, G.F. Chavez, H.K. Atrash, D.J. Taylor, R.S. Shah, D. Rowley, Hospitalizations for severe complications of pregnancy, Obstet. Gynecol. 90 (1997) 225–229.
[59] R.B. Ness, B.M. Sibai, Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia, Am. J. Obstet. Gynecol. 195 (2006) 40–49.
[60] R.J. Levine, M.D. Lindheimer, First-trimester prediction of early preeclampsia: a possibility at last, Hypertension 53 (5) (2009) 747–748.
[61] R.B. Ness, J.M. Roberts, Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications, Am. J. Obstet. Gynecol. 175 (1996) 1365–1370.
[62] S.E. Maynard, J.Y. Min, J. Merchan, K.H. Lim, J. Li, S. Mondal, T.A. Libermann, J.P. Morgan, F.W. Selke, I.E. Stillman, F.H. Epstein, V.P. Sukhatme, S.A. Karumanchi, Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia, J. Clin. Invest. 111 (2003) 649–658.
[63] R.J. Levine, S.E. Maynard, C. Qian, B.M. Sibai, V.P. Sukhatme, S.A. Karumanchi, Circulating Angiogenic factors and the risk of preeclampsia, N. Engl. J. Med. 350 (7) (2004) 672–683.
[64] G. Wallukat, V. Homuth, T. Fischer, C. Lindschau, B. Horstkamp, A. Jüpner, E. Baur, E. Nissen, K. Vetter, D. Neichel, J.W. Dudenhausen, H. Haller, F.C. Luft, Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT_1 receptor, J. Clin. Invest. 103 (7) (1999) 945–952.
[65] L. Poston, Sodium transport inhibitors in pregnancy-induced hypertension, Cardiovasc. Drugs Ther. 4 (1990) 351–356.
[66] R.C. Goodlin, Antidigoxin antibodies in eclampsia, N. Engl. J. Med. 318 (8) (1988) 518–519.
[67] D.E. Scott, Anemia in pregnancy, Obstet. Gynecol. Annu. 1 (1972) 219–244.
[68] M. Ianosi-Irimie, J.D. Nadig, M.W. Williams, C.A. Pridjian, J.M. Whitbred, H.V. Vu, D.C. Wrenn, G. Pridjian, J.B. Puschett, A rat model of preeclampsia, Clin. Exp. Hypertens. 8 (2005) 605–617.
[69] A.B. Alper, J. Outland, K. Finigan, G. Pridjian, A. Aina-Mumuney, J.B. Puschett, Phenotypic characteristics shared by preeclamptic patients and an animal model of the syndrome: report of a pilot study, Am. J. Hypertens. 19 (9) (2006) 947–950.
[70] G. Pridjian, C. Pridjian, S. Danchuk, M. Ianosi-Irimie, H.V. Vu, J.B. Puschett, Beneficial effects of metolazone in a rat model of preeclampsia, J. Pharmacol. Exp. Ther. 318 (2006) 1027–1032.

[71] E.R. Norwitz, D.J. Schust, S.J. Fisher, Implantation and the survival of early pregnancy, N. Engl. J. Med. 345 (19) (2001) 1400–1408.
[72] R. Pijnenborg, W.B. Robertson, I. Brosens, G. Dixon, Review article: trophoblast invasion and the establishment of haemochorial placentation in man and laboratory animals, Placenta 2 (1) (1981) 71–91.
[73] R. Pijnenborg, J.M. Bland, W.B. Robertson, G. Dixon, I. Brosens, The pattern of interstitial trophoblastic invasion of the myometrium in early human pregnancy, Placenta 2 (4) (1981) 303–316.
[74] H.L. Lamarca, C.A. Morris, G.R. Pettit, T. Nagowa, J.B. Puschett, Marinobufagenin impairs first trimester cytotrophoblast differentiation, Placenta 27 (2006) 984–988.
[75] S. Weiss, L. Dexter, Preeclamptic and Eclamptic Toxemia of Pregnancy, Williams & Wilkins, Baltimore, 1941, pp. 168–327.
[76] J.M. Roberts, R.N. Taylor, T.J. Musci, G.M. Rodgers, C.A. Hubel, M.K. McLaughlin, Preeclampsia: an endothelial cell disorder, Am. J. Obstet. Gynecol. 161 (1989) 1200–1204.
[77] H.M. Silver, M. Seebeck, R. Carlson, Comparison of total blood volume in normal, preeclamptic, and nonproteinuric gestational hypertensive pregnancy by simultaneous measurement of red blood cell and plasma volumes, Am. J. Obstet. Gynecol. 179 (1998) 87–93.
[78] M.A. Brown, V.C. Zammit, S.A. Lowe, Capillary permeability and extracellular fluid volumes in pregnancy-induced hypertension, Clin. Sci. 77 (1989) 599–604.
[79] M.N. Uddin, L.B. McLean, F.A. Hunter, D. Horvat, J. Severson, B. Tharakan, E.W. Childs, J.B. Puschett, Vascular leak in a rat model of preeclampsia, Am. J. Nephrol. 30 (2009) 26–33.
[80] B.M. Mitchell, L.G. Cook, S. Danchuk, J.B. Puschett, Uncoupled endothelial nitric oxide synthase and oxidative stress in a rat model of pregnancy-induced hypertension, Am. J. Hypertens. 20 (2007) 1297–1304.
[81] K. Kanasaki, R. Kalluri, The biology of preeclampsia, Kidney Int. 76 (2009) 831–837.
[82] G.G. Zeeman, Neurologic complications of pre-eclampsia, Semin. Perinatol. 33 (2009) 166–172.
[83] E. Agunanne, M.N. Uddin, D. Horvat, J.B. Puschett, Angiogenic factors in a rat model of preeclampsia, Hypertension 54 (4) (2009) e26–e127 (e109 (P362)).
[84] M.N. Uddin, E.E. Agunanne, D. Horvat, J.B. Puschett, Marinobufagenin causes enhanced permeability in human brain microvascular endothelial cells via apoptotic signaling, J. Am. Soc. Nephrol. 20 (2009) 534A–535A (F-PO1847).
[85] K.A. Okanloma, J. Moodley, Neurological complications associated with the pre-eclampsia/eclampsia syndrome, Int. J. Gynecol. Obstet. 71 (2000) 223–225.
[86] M.J. Cipolla, Cerebrovascular function in pregnancy and eclampsia, Hypertension 50 (2007) 14–24.
[87] A.G. Euser, M.J. Cipolla, Cerebral blood flow autoregulation and edema formation during pregnancy in anesthetized rats, Hypertension 49 (2007) 334–340.
[88] M.J. Cipolla, N. DeLance, L. Vitullo, Pregnancy prevents hypertensive remodeling of cerebral arteries, Hypertension 47 (Pt 2) (2006) 619–626.
[89] A.Y. Bagrov, O.V. Fedorova, R.I. Dmitrieva, W.N. Howald, A.P. Hunter, E.A. Kuznetsova, V.M. Shpen, Characterization of a urinary bufodienolide Na^+, K^+-ATPase inhibitor in patients after acute myocardial infarction, Hypertension 31 (1998) 1097–1103.
[90] A.Y. Bagrov, O.V. Fedorova, J.L. Austin-Lane, R.I. Dmitrieva, D.E. Anderson, Endogenous marinobufagenin-like immunoreactive factor and Na^+, K^+ ATPase inhibition during voluntary hypoventilation, Hypertension 26 (1995) 781–788.