The Ouabain-Binding Site of the α2 Isoform of Na,K-ATPase Plays a Role in Blood Pressure Regulation During Pregnancy

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BACKGROUND

The cardiotonic steroid/ouabain-binding site of the α subunit of Na,K-ATPase is thought to play an important role in cardiovascular homeostasis. Previously, we demonstrated the cardiotonic steroid-binding site of the α2 Na,K-ATPase is involved in adrenocorticotropic hormone (ACTH)–induced hypertension by using gene-modified α2R/R mice in which the cardiotonic steroid–binding site is relatively resistant to ouabain compared to the ouabain-sensitive wild-type α2S/S mice. To further explore the importance of this site in the cardiovascular system, we investigated blood pressure regulation during pregnancy in mice with the α2R/R isoform.

METHODS

The systolic blood pressure (SBP) of the α2S/S and α2R/R mice was measured before and during pregnancy by tail-cuff. The expression of the α isoforms of Na,K-ATPase in various tissues and plasma endogenous ouabain contents were assessed prior to pregnancy as well as days 7 and 17 of gestation.

RESULTS

The α2S/S mice showed a gradual decrease in the SBP during the first two trimesters, followed by an increase above the preconceptional level in the third trimester. However, the α2R/R mice exhibited a lower blood pressure in the third trimester. The cardiac expression of the α2 Na,K-ATPase in the α2S/S mice was significantly less than that of the α2R/R mice throughout the pregnancy. The plasma endogenous ouabain concentration significantly increased by twofold at day 17 of pregnancy in the α2R/R mice but not in the α2S/S mice.

CONCLUSIONS

The cardiotonic steroid–binding site of the α2 Na,K-ATPase plays a role in maintaining normal SBP during pregnancy.

Keywords: α2 Na,K-ATPase; blood pressure; cardiotonic steroid; conception; hypertension; ouabain

American Journal of Hypertension, advance online publication 9 September 2010, doi:10.1038/ajh.2010.195

The four catalytic α isoforms of Na,K-ATPase, α1, α2, α3, and α4, have been identified, exhibiting unique tissue distribution, substrate affinity, and physiological roles.1,2 The cardiotonic steroid/ouabain-binding site in the α subunit of Na,K-ATPase is highly conserved among different species, suggesting physiological importance of this binding site. In order to study the significance of the ouabain-binding site of α2 Na,K-ATPase, we developed genetically modified mice with the mutations of L111R and N122D, designated as the α2R/R mice that are relatively resistant to ouabain compared to the ouabain-sensitive wild-type α2S/S mice.3 We previously demonstrated the ouabain-binding site of the α2 Na,K-ATPase is involved in adrenocorticotropic hormone (ACTH)–induced hypertension.4,5 The α2S/S mice developed hypertension after 5 days of ACTH treatment, whereas the α2R/R mice did not exhibit an increase in blood pressure. This suggests that during ACTH treatment, endogenous cardiotonic steroids are released and interact with the ouabain-sensitive cardiotonic steroid–binding site in the α2S/S mice. Moreover, there is some evidence that endogenous cardiotonic steroids regulate cardiovascular function, renal homeostasis, and homeostasis by their direct interaction with the α subunit of Na,K-ATPase.3,6–10

Normal pregnancy involves a number of maternal physiological changes to meet the demands of fetal development.11,12 Particularly, a continuous increase in extracellular fluid and plasma volume, and a significant decrease in systemic vascular resistance are essential physiological adaptations that begin in the first trimester.13–15 The gestational plasma volume expansion is characterized by renal Na⁺ and fluid retention. During normal pregnancy, natriuresis in response to atrial natriuretic peptide is remarkably reduced in rats16 and goats.17,18 The upregulation of aquaporin 2, a water channel expressed in the renal collecting duct, and a reduction of the osmotic thresholds for the vasopressin secretion and the perception of thirst during gestation also result in increased water intake and retention.14,19,20 Interestingly, in normotensive human pregnancy, atrial natriuretic peptide maintains its natriuretic effect, suggesting species differences in the renal response to atrial natriuretic peptide after conception.21 Despite the substantial volume expansion during pregnancy, blood pressure

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Received 11 March 2010; first decision 6 April 2010; accepted 5 August 2010.

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of pregnant females is significantly lower compared to their preconceptional blood pressure.\textsuperscript{22–24} This is attributed to peripheral vasodilation causing a considerable fall in systemic vascular resistance. As a result of the gestational decline in systemic vascular resistance, a compensatory increase in cardiac output is profound in both mid- and late gestation compared to nonpregnant animals.\textsuperscript{25}

The physiological significance of cardiotonic steroids in the cardiovascular system during pregnancy has been implicated in the pathogenesis of a pregnancy-induced hypertensive disorder, pre-eclampsia. Pre-eclampsia is characterized by the onset of hypertension and proteinuria after midgestation, and a leading cause of maternal and fetal morbidity and mortality, affecting 3–8\% of all pregnancies.\textsuperscript{26} In pre-eclampsia rats and human subjects, renal excretion of a cardiotonic steroid, marinobufagenin, and plasma marinobufagenin level increased remarkably compared to the normal pregnancy.\textsuperscript{22,27} Administration of the antimarinobufagenin antibody to pre-eclamptic animals and humans restores the Na,K-ATPase activity in thoracic aorta and erythrocyte, respectively.\textsuperscript{22,27} Moreover, the elevated systolic blood pressure (SBP) is decreased to the normal pregnancy level when the antibody was administered.\textsuperscript{22,27} This suggests that the cardiotonic steroid–binding site of Na,K-ATPase might be involved in the regulation of blood pressure during pregnancy. The main goal of this study was to investigate the physiological role of the ouabain-binding site of the \(\alpha_2\) Na,K-ATPase in gestational blood pressure regulation.

\section*{METHODS}

\subsection*{Animals}
Mice expressing the ouabain-resistant \(\alpha_2^{R/R}\) Na,K-ATPase were generated by gene targeting as described previously.\textsuperscript{3} Female wild-type \(\alpha_2^{SS}\) mice and \(\alpha_2^{R/R}\) littermates were 3–4 months of age and maintained on a mixed background of 129SvJ and Black Swiss. All mice were kept in a 12-h light–dark cycle and temperature controlled room with access to regular rodent diet (Harlan Teklad, Madison, WI) and tap water \textit{ad libitum}. Genotypes of the mice were determined by PCR using genomic DNA from tail biopsies.\textsuperscript{3} All procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

\subsection*{Blood pressure measurements}
SBP of conscious animals was measured by tail-cuff using a Visitech System (Apex, NC) as described previously.\textsuperscript{8}

\subsection*{Plasma endogenous cardiotonic steroids}
The concentration of plasma ouabain was measured by competitive fluorescence immunoassay as described previously\textsuperscript{22} with modification. Briefly, plasma was collected from mice before conception and days 7 and 17 of gestation. At least two plasma samples in the same experimental group were pooled and extracted using Sep-Pak C18 cartridges (Waters, Milford, MA). The eluent was vacuum-dried and reconstituted in one tenth of the original plasma volume. 0.05\(\mu\)g ouabain–thyroglobulin conjugate (kindly provided by Alexei Y. Bagrov, NIH) was plated on 96-well plates and incubated for 17\,h. After blocking the plates with 3% nonfat milk, 20\(\mu\)l of the plasma extracts or ouabain standards were applied, followed by 100\(\mu\)l of a rabbit antiouabain antibody (1:8,000, kindly provided by Alexei Y. Bagrov, NIH). Subsequently, 100\(\mu\)l of europium-labeled anti-rabbit IgG (1:2,000, PerkinElmer, Waltham, MA) was added. Fluorescence derived from europium was detected by FLUOstar Optima (BMG Labtech, Cary, NC).

\subsection*{Western blot analysis}
Brain, heart, aorta, and kidney were isolated from anesthetized mice and quickly frozen in liquid nitrogen. For aorta samples, they were pooled from at least four mice in the same experimental group. The frozen tissues were homogenized in a buffer containing 10\,mmol/l Tris, 1\,mmol/l dithiothreitol, and 0.25\% Igepal CA-630, supplemented with protease inhibitor cocktail (Sigma, St Louis, MO) and phosphatase inhibitor cocktail I and II (Sigma) at ratio of 1:100. Protein concentration was measured by bicinchoninic acid protein assay kit (Thermo Scientific, Rockford, IL). Protein samples were denatured and resolved by 8\% SDS-polyacrylamide gels. The primary antibodies used were an \(\alpha_1\) Na,K-ATPase monoclonal antibody (1:2,000, a6f; University of Iowa Developmental Hybridoma Bank, Iowa City, IA), an \(\alpha_2\) Na,K-ATPase anti-HERED polyclonal antibody\textsuperscript{28} (1:500), and an \(\alpha_3\) Na,K-ATPase monoclonal antibody (1:1,000, MA3-915; Affinity Bioreagents, Golden, CO). The signal was detected using Supersignal West Pico chemiluminescent substrate kit (Thermo Scientific). The densitometric analysis of the target protein bands was performed utilizing Image-Quant software (Molecular Dynamics, Sunnyvale CA). For a loading control, GAPDH was detected using anti-GAPDH monoclonal antibody (1:2,000; Cell Signaling Technology, Beverly, MA).

\subsection*{Data analysis}
Data are shown as means ± s.e.m. Using the SigmaStat 3.5 (Systat Software, San Jose, CA), statistical analysis was performed by one- and two-way repeated measures analysis of variance for intragroup and intergroup analysis, respectively, in \textbf{Figure 1}. \textbf{Figures 2–4} and \textbf{Table 3} were analyzed by two-way analysis of variance. \textit{Post hoc} test used to compare individual group means was Holm–Sidak test, and differences were considered to be statistically significant at \(P < 0.05\).

\section*{RESULTS}

\subsection*{Delivery outcome}
To further explore the physiological significance of the cardiotonic steroid/ouabain-binding site of the \(\alpha_2\) Na,K-ATPase, we first investigated changes in maternal body weight during pregnancy as shown in \textbf{Table 1}. Both the \(\alpha_2^{SS}\) and \(\alpha_2^{R/R}\) mice gradually increased in the body weight until day 10 of gestation, and the increments became pronounced in the third trimester, with a total weight gain of about 20\,g. After delivery, the body weight of the \(\alpha_2^{SS}\) and \(\alpha_2^{R/R}\) mice recovered to the preconceptional level. Moreover, as shown in \textbf{Table 2}, there is no statistical difference in the term of gestation or the size of the newborns between the two genotypes.
SBP during pregnancy in the α2<sup>S/S</sup> and α2<sup>R/R</sup> mice
The SBP was measured in the α2<sup>S/S</sup> and the α2<sup>R/R</sup> mice by tail-cuff. As shown in Figure 1, no significant effect of the genotype was found on the preconceptional SBP between the α2<sup>S/S</sup> and the α2<sup>R/R</sup> mice, 126.6 ± 2.5 and 119.5 ± 2.9 mm Hg, respectively. This is consistent with our previous studies using tail-cuff<sup>5,4,8</sup> as well as telemetry.<sup>5</sup> The wild-type α2<sup>S/S</sup> mice showed a gradual decrease in the SBP during the first two trimesters, reaching the lowest level on day 9 of pregnancy, 116.7 ± 2.5 mm Hg, followed by the elevation of the SBP in the third trimester (130.7 ± 3.0 mm Hg on day 18 of gestation). This pregnancy-associated change in SBP has also been reported previously with rats<sup>22</sup> and mice.<sup>24,29</sup> Similarly, during the first and second trimesters, the α2<sup>R/R</sup> mice exhibited a decline of SBP from the baseline to 108.5 ± 2.8 mm Hg on day 8 of gestation. However, unlike the α2<sup>S/S</sup> mice, the SBP did not rise in the third trimester, which measured 117.8 ± 3.7 mm Hg on gestation day 18. The SBP in the α2<sup>R/R</sup> mice was significantly lower than that in the α2<sup>S/S</sup> mice.

Figure 1 | The systolic blood pressure (SBP) measurements in the ouabain-sensitive wild-type (α2<sup>S/S</sup>) and the ouabain-resistant α2 (α2<sup>R/R</sup>) mice during pregnancy. Prior to mating, mice were acclimated for 6 days, followed by baseline (BS) systolic blood pressure measurements for three consecutive days. After the baseline recordings, the female mice were combined with the wild-type α2<sup>S/S</sup> male mice. The morning of the date when copulation plugs were found was designated as day 0 of gestation. The systolic blood pressure was measured every morning until delivery. The computerized measuring session consisted of five readings for acclimation followed by 15 continuous recordings of systolic blood pressure. The session was considered valid when systolic blood pressure was identified by the computer in at least 8 of the 15 measurements. The data shown are mean ± s.e.m., five readings for acclimation followed by 15 continuous recordings of systolic blood pressure. The session was considered valid when systolic blood pressure was identified by the computer in at least 8 of the 15 measurements. The data shown are mean ± s.e.m., n = 18 mice (α2<sup>S/S</sup>) and n = 17 mice (α2<sup>R/R</sup>). *Statistical significance compared with the corresponding value in the α2<sup>S/S</sup> mice, P < 0.05. #,‡ P < 0.05 vs. BS in the α2<sup>S/S</sup> and the α2<sup>R/R</sup> mice, respectively.

Figure 2 | The expression of the α1 isoform of Na,K-ATPase in brain, heart, and kidney before and during pregnancy. a–c are representative immunoblots for the Na,K-ATPase α1 isoform and GAPDH in brain, heart, and kidney, respectively. Each tissue was collected from the ouabain-sensitive α2<sup>S/S</sup> and the ouabain-resistant α2<sup>R/R</sup> mice before conception for baseline and at days 7 and 17 of pregnancy. The amount of protein loaded per lane is 2 µg for brain, 5 µg for heart, and 2.5 µg for kidney. d–f are measurements by densitometry. The values were obtained from four independent immunoblots and normalized with the amount of corresponding GAPDH. The abundance of the α1 Na,K-ATPase is expressed as a relative ratio to that in the wild-type α2<sup>S/S</sup> mice at baseline (open bars). The bars represent mean ± s.e.m., n = 8 mice except the α2<sup>S/S</sup> baseline and day 7 and α2<sup>R/R</sup> baseline in kidney, n = 7 mice. *Statistical significance, P < 0.05.
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mice on days 1–4, 6–8, and 14–18. To verify the changes in SBP is associated with pregnancy, we also measured SBP of non-pregnant α2S/S and α2R/R mice. In our preliminary experiments, the SBP of the α2S/S and α2R/R mice did not change or differ between the two genotypes subsequent to the formation of copulation plugs if they did not conceive (data not shown). These results demonstrate blood pressure is regulated differently in the α2S/S and α2R/R mice after conception, although the cardiovascular function of the α2R/R mice remains intact under the resting conditions. This suggests that the ouabain-binding site in the α2 Na,K-ATPase plays a role in maintaining the normal blood pressure under a physiologically relevant stress condition such as pregnancy.

The expression of the α1, α2, and α3 isoforms of Na,K-ATPase during pregnancy

In order to investigate mechanisms resulting in the pregnancy-induced difference in SBP between the α2S/S and α2R/R mice, the level of the α1, α2, and α3 isoforms in brain, heart, and kidney was examined in the α2S/S and α2R/R mice (Figures 2 and 3). Representative immunoblots for the α1 isoform of Na,K-ATPase in the target tissues are shown in Figure 2a–c, and relative expression level of the isoform is shown in Figure 2d–f. The abundance of the α1 isoform in brain showed an insignificantly increase at days 7 and 17 of gestation in both the α2S/S and α2R/R mice (Figure 2a,d), and no pregnancy-associated change in the expression of the α1 isoform was observed in the heart of either genotype of animals (Figure 2b,e). However, the renal α1 expression in the wild-type mice decreased by 40% at day 7 of gestation and recovered to the preconceptional level at day 17 (Figure 2c,f). This is consistent with a previous report in which the α1 expression in the renal cortex from midpregnant rats is reduced by 50% that of the virgin rats. The α2R/R mice exhibited the same trend by decreasing the α1 expression at day 7 (~30%) and significantly increasing at day 17. This phenomenon might be part of a physiological regulatory mechanism of extracellular volume expansion and Na+ retention during pregnancy in cooperation with other membrane transporters in kidney.

The expression of the α2 isoform of Na,K-ATPase was also examined in several tissues prior to and during pregnancy (Figure 3a–d). As shown in Figure 3a,d, there was no pregnancy-induced change or effect of the genotype on the abundance of the α2 Na,K-ATPase in brain from the α2S/S and α2R/R mice. This result is consistent with a previous finding that the expression of the α2 Na,K-ATPase in the brain stem did not change during pregnancy. In heart, however, the wild-type α2S/S mice exhibited a 30% decrease in the expression of the α2 Na,K-ATPase at day 7 of pregnancy and a 40% reduction at day 17, whereas the α2R/R mice showed a 20% decrease at day 7 and recovered to the baseline at day 17 (Figure 3b,e). Moreover, throughout the gestation, the cardiac expression of the α2 Na,K-ATPase was significantly higher in the α2R/R mice than the α2S/S mice. No detectable bands for the α2 Na,K-ATPase were found in either genotype of animals in kidney (data not shown).

The expression of the α3 isoform of Na,K-ATPase in brain is shown in Figure 3c,f. There was no significant effect of the genotypes or pregnancy on the abundance of the α3 isoform of Na,K-ATPase.
α3 Na,K-ATPase, although a minor reduction at day 7 of gestation was observed in both genotypes of animals. Cardiac and renal expressions of the α3 Na,K-ATPase were not detected by western blot (data not shown).

Because the vasculature plays an important role in regulating blood pressure, the expression of the α1, α2, and α3 isoforms in aorta was examined. As shown in Figure 4a,c, in the wild-type α2S/S mice, the aortic expression of the α1 Na,K-ATPase showed a 1.7-fold increase at day 7 of pregnancy and was significantly reduced by 66% of the day 7 level at day 17. The α2R/R mice exhibited similar changes: the expression of the α1 Na,K-ATPase at day 7 of gestation was significantly increased 2.6-fold of the basal abundance and decreased to the baseline level at day 17. The increased aortic expression of the α1 Na,K-ATPase at day 7 in the α2S/S and α2R/R mice corresponds to a pregnancy-induced decrease in SBP in the early pregnancy. Unlike the α1 isoform, the abundance of the α2 Na,K-ATPase in aorta did not significantly change at day 7 of pregnancy in either genotype of animals (Figure 4b,d). However, the expression of the α2 Na,K-ATPase significantly decreased at day 17 by 75 and 50% of the day 7 values in the α2S/S and α2R/R mice, respectively. No effect of the genotypes was seen on the abundance of the α1 and α2 isoforms in aorta. Also, the α3 Na,K-ATPase was undetectable by western blot in aorta (data not shown).
Plasma endogenous ouabain during pregnancy

The plasma concentration of endogenous ouabain during pregnancy was measured using the polyclonal antiouabain antibody (Table 3). In the wild-type α2S/S mice, the level of plasma endogenous ouabain did not significantly change throughout pregnancy, 0.017 ± 0.002 nmol/l before conception, 0.018 ± 0.003 nmol/l at day 7, and 0.013 ± 0.001 nmol/l at day 17 of pregnancy. However, the α2R/R mice showed a twofold increase in plasma endogenous ouabain at day 17 of gestation compared to the preconceptional value, 0.046 ± 0.014 nmol/l and 0.022 ± 0.003 nmol/l, respectively.

DISCUSSION

The goal of this study was to investigate the physiological role of the cardiotonic steroid/ouabain-binding site of the α2 Na,K-ATPase in blood pressure regulation during pregnancy. Although the tail-cuff induces some stress, we found that the α2 R/R mice maintained a significantly lower SBP in the third trimester than the wild-type α2 S/S mice. This indicates that the ouabain-binding site of the α2 Na,K-ATPase plays a role in maintaining normal blood pressure in late pregnancy. Moreover, a significant increase in plasma endogenous ouabain was detected at day 17 of gestation in the α2 R/R mice but not in the α2 S/S mice. Because the plasma endogenous ouabain cannot interact with the ouabain-resistant α2 R/R Na,K-ATPase, this increase could result from compensatory upregulation of endogenous ouabain production in the α2 R/R mice in an attempt to restore the SBP in the third trimester.

It was unexpected to find that the expression of the α2 Na,K-ATPase in heart was different between the α2 S/S and α2 R/R mice during pregnancy, and we are not sure how changing the affinity for ouabain in the α2 Na,K-ATPase is associated with the change in the expression of the α2 Na,K-ATPase. One potential explanation for this observation is that the amino acid substitutions at positions 111 and 122 to develop the ouabain-resistant α2 R/R isoform might have disrupted ouabain-induced signaling for endocytosis and protein degradation in the intracellular compartments. It has been demonstrated that ouabain binding to Na,K-ATPase expressed on the plasma membrane of HeLa cells initiates internalization of the ouabain–Na,K-ATPase complex into the cells and results in translocation of the complex to the lysosome for degradation.31–33 Moreover, in LLC-PK1 cells, a pig renal proximal tubule cell line, low concentration of ouabain plays a role in the clathrin-dependent endocytosis of Na,K-ATPase.34,36 Because the α2 R/R Na,K-ATPase cannot function as a signal transducer for endogenous ouabain, in the ouabain-resistant α2 R/R mice, not only would the ouabain-triggered endocytosis pathway but also the following lysosomal protein degradation pathway be perturbed. Therefore, the difference between the α2 S/S and α2 R/R mice in the expression of the α2 Na,K-ATPase in heart might be due to a disrupted ouabain-evoked signaling for endocytosis and protein degradation. However, because the expression of the α2 Na,K-ATPase was examined using the whole heart homogenates in this study, the subcellular distribution of the α2 Na,K-ATPase is ambiguous. As a result, the increase in the α2 Na,K-ATPase in the heart of the α2 R/R mice is not necessarily on the sarcolemmal membrane. An increased production of the cardiac α2 Na,K-ATPase in the α2 R/R mice is also a possibility, but real-time PCR revealed that there is no profound difference between the α2 S/S and α2 R/R mice in the expression of the α2 Na,K-ATPase mRNA in heart (data not shown).

The physiological significance of the cardiotonic steroids and their binding site in blood pressure regulation has been studied by our laboratory and others using different hypertensive models. Previously, we have shown that the α2 R/R mice are resistant to ACTH-induced hypertension, which is often considered as a hypertension model correlated to stress.4 Moreover, in a volume-dependent form of hypertension, the blood pressure of deoxycorticosterone acetate–salt treated rats was reduced when the circulating cardiotonic steroids were sequestered by Digibind.38 The cardiotonic steroids, especially marinobufagenin, are implicated in the pathogenesis of pre-eclampsia, which is a pregnancy-associated hypertensive disorder. In the pre-eclampsia model of animals and humans, renal excretion and plasma level of marinobufagenin increased profoundly compared to the normotensive pregnancy.22,27 In addition, administration of the antimarinobufagenin antibody to pre-eclamptic animals and humans rescued the activity of Na,K-ATPase, resulting in a reduction of the elevated SBP to the normal level. These reports further support the physiological importance of the cardiotonic steroid–binding site of Na,K-ATPase in cardiovascular system.

Further studies are necessary to elucidate a mechanism of our present observation in which the α2 R/R mice exhibit lower blood pressure in the late pregnancy compared to the α2 S/S mice. Our study using pregnancy as another stress-related condition provides evidence of physiological importance of the cardiotonic steroid/ouabain-binding site in regulation of blood pressure.

Acknowledgment: Many thanks to Alexei Y. Bagrov for providing valuable technical support and reagents used in the fluoroimmunoassay. We also thank Maureen L. Bender for animal husbandry. This research was supported by National Institutes of Health grant R01 HL28573 and R01 HL66062.

Disclosure: The authors declared no conflict of interest.

6. Dostanic-Larson I, Lorenz JN, Van Huyse JW, Neumann JC, Moseley AE, Lingrel JB. Physiological role of the alpha 1- and alpha 2-isoforms of the Na+—K+—ATPase and their binding site in blood pressure regulation has been studied by our laboratory and others using different hypertensive models. Previously, we have shown that the α2R/R mice are resistant to ACTH-induced hypertension, which is often considered as a hypertension model correlated to stress.4 Moreover, in a volume-dependent form of hypertension, the blood pressure of deoxycorticosterone acetate–salt treated rats was reduced when the circulating cardiotonic steroids were sequestered by Digibind.38 The cardiotonic steroids, especially marinobufagenin, are implicated in the pathogenesis of pre-eclampsia, which is a pregnancy-associated hypertensive disorder. In the pre-eclampsia model of animals and humans, renal excretion and plasma level of marinobufagenin increased profoundly compared to the normotensive pregnancy.22,27 In addition, administration of the antimarinobufagenin antibody to pre-eclamptic animals and humans rescued the activity of Na,K-ATPase, resulting in a reduction of the elevated SBP to the normal level. These reports further support the physiological importance of the cardiotonic steroid–binding site of Na,K-ATPase in cardiovascular system.

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biological significance of their cardiac glycoside binding site. Am J Physiol Regul Integr Comp Physiol 2006; 290:R524–R528.


