Differential Regulation of the Sodium Pump $\alpha$-Subunit Isoform Gene by Ouabain and Digoxin in Tissues of Rats

Hao WANG, Wei-Qing YUAN, and Zhuo-Ren LU

The effects of ouabain and digoxin on both the systolic blood pressure (SBP) and sodium pump $\alpha$-subunit expression in some tissues of rats were compared. Normal rats were injected with ouabain, digoxin, and normal saline (NS), respectively, everyday, and indirect SBP was recorded once a week. Six weeks later, all the rats were killed, and sodium pump $\alpha_1$-, $\alpha_2$-, and $\alpha_3$-subunit mRNA levels were detected in the myocardium, kidney, adrenal gland, aortic smooth muscle, and hypothalamus by the RT-PCR method. The results showed that the SBP of rats infused with ouabain increased significantly at the end of week 6, while no difference in SBP was found between the digoxin and NS groups. The effects of ouabain and digoxin on sodium pump $\alpha$-subunit isoform expression were also different. Myocardium: both ouabain and digoxin stimulated expression of the $\alpha_3$-isoform whereas $\alpha_2$ was unchanged. Levels of the $\alpha_1$ isoform decreased significantly in the ouabain group and decreased slightly in the digoxin group, respectively. Kidney: digoxin had the same effects as ouabain. $\alpha_1$ levels increased, but those of $\alpha_2$ and $\alpha_3$ remained unchanged. Adrenal gland: $\alpha_2$ and $\alpha_3$ levels increased, but those of $\alpha_1$ decreased in the ouabain group. $\alpha_1$ and $\alpha_3$ levels increased and those of $\alpha_2$ remained unchanged in the digoxin group. Aortic smooth muscle: both ouabain and digoxin increased $\alpha_1$ and $\alpha_3$ expression. $\alpha_2$ levels decreased in the digoxin group but remained unchanged in the ouabain group. Hypothalamus: both ouabain and digoxin stimulated $\alpha_1$ expression, while $\alpha_2$ and $\alpha_3$ levels remained unchanged. The results of this study have shown that ouabain and digoxin have the different effects on both the systolic blood pressure and expression of sodium pump $\alpha$-subunit isoforms in some tissues in rats. Further studies on the expression of sodium pump $\alpha$-subunit isoforms might be helpful for the understanding of the physiological role of endogenous ouabain and the molecular mechanisms involved in the pathogenesis of hypertension.

(Hypertens Res 2000; 23 Suppl: S55-S60)

Key Words: ouabain, digoxin, sodium pump, hypertension, rat

Introduction

Accumulating evidence indicates that endogenous ligands of the digitalis receptor, which have recently been reported to be indistinguishable from ouabain, may exist in the mammalian body (1, 2). Endogenous ouabain (EO) might induce many cytobiological changes and play an important role in regulating water and sodium metabolism and vascular tone in the body (3, 4). Many studies have shown that the EO content in both hypertensive patients and hypertensive animals is much greater than that of normal subjects, suggesting that higher EO levels might be involved in the development of hypertension (5-7). Hamlyn and colleagues (8, 9) have reported that the administration of ouabain to either Wistar rats or
Sprague-Dawley (SD) rats can induce hypertension. However, the mechanism by which ouabain induces hypertension is not completely clear. It has been suggested that EO induces a series of changes with cytobiological effects and leads to hypertension by changing the sodium pump (Na⁺,K⁺-ATPase) configuration and inhibiting its activity, and that this might be the only effect of EO on the sodium pump according to traditional views (3, 4).

The sodium pump, acting as a “receptor” for cardiac glycosides such as digoxin and ouabain, consists of genes encoding for at least three α-subunit isoforms and two β-subunit isoforms. The α-subunit contains the catalytic and ouabain binding sites and possesses specialized functions, and the β-subunit is a glycoprotein that is essential for the normal function and assembly of the Na⁺,K⁺-ATPase (10). These isoforms are expressed in a tissue- and cell-specific fashion, and are controlled by developmental and hormonal regulatory influences (10-12). In addition, this gene expression can be affected in some diseased conditions such as hypertension and a hypertrophied myocardium (13, 14). But the effects of ouabain on sodium pump α-subunit gene expression is not well understood. This study was designed to investigate the effects of ouabain on sodium pump α-subunit gene expression in vivo and to compare these effects with those of digoxin.

**Materials and Methods**

**Ouabain and Digoxin Infusion and Blood Pressure Measurement**

Twenty-four healthy adult male SD rats (6-10 weeks old) weighing 200-250 g were purchased from the Experimental Animal Center of Xi'an Medical University. They were given free access to tap water and standard rat chow. After 1 week, the body weight and systolic blood pressures of the rats were determined to document normotension. The rats were divided randomly into three groups: the ouabain group (O group, n=8), digoxin group (D group, n=8) and normal saline group (N group, n=8). Animals in these three groups were given ouabain (Sigma Chemical Co., 20 µg · kg⁻¹ · d⁻¹) (8, 9), digoxin (Sigma Chemical Co., 32 µg · kg⁻¹ · d⁻¹), and normal saline (1 ml · kg⁻¹ · d⁻¹) intraperitoneally daily, respectively. Feeding and living conditions were the same among these groups throughout the study. Indirect systolic blood pressure (SBP) was recorded by the tail cuff method once a week using the Heart Rate & Blood Pressure Recorder for Rats (Model MRB-IIIA, Shanghai Hypertension Institute, Shanghai, China). At the end of week 6, all of the rats were fasted overnight and killed by decapitation on the following morning. The left ventricular myocardium, cortex of the kidney, adrenal gland, thoracic aortic smooth muscle, and hypothalamus were collected for detecting mRNA levels of the sodium pump α-subunit isoform using reverse transcription polymerase chain reaction (RT-PCR) techniques. Those procedures were in accordance with the institutional and the National Research Council’s guidelines for animal experiments.

**RT-PCR**

Total RNA was extracted from the above tissues as previously described (12), followed by digestion with 5 U RNase-free DNase (Boehringer Mannheim, Germany). Total RNA, 1 µg, was used to synthesize the first strand of cDNA. RNA samples were heated to 95°C for 4 min, chilled on ice, and reverse-transcribed at 48°C for 45 min in 23 µl containing 10 pmol reverse primer, 200 U reverse transcriptase, 400 µmol/l of each deoxynucleotide triphosphate (dNTP: dATP, dTTP, dCTP, and dGTP), 50 mmol/l Tris-HCl (pH 8.3), 75 mmol/l KCl, and 3 mmol/l MgCl₂. The reaction was terminated by heating to 65°C for 10 min. Equal volumes of resultant product were amplified in a final volume of 50 µl containing the following: 200 µmol/l dNTP, 5 pmol each of forward and reverse primers, 10 mmol/l Tris-HCl (pH 8.8), 50 mmol/l KCl, 1.5 mmol/l MgCl₂, 0.1% TritonX 100, and 2.5 U Taq polymerase (Promega, Madison, WI). Samples were denatured at 94°C for 2 min and cycled 25 times through the following steps: 1 min at 94°C, 1 min at 52°C, and 2 min at 68°C. The final cycle was extended for 7 min at 68°C. PCR products were electrophoresed on 2% agarose gels and quantitated by scanning densitometry (Gelworks ID Intermediate 3.01). RT-PCR of the glyceraldehyde-3-phosphate-dehydrogenase (G3PDH) gene was performed.

**Table 1. Primer Sequences for Amplifying Sodium Pump α₁-, α₂-, α₃-Subunit Isoforms and G3PDH**

<table>
<thead>
<tr>
<th>Primer specificity</th>
<th>Position of bp in cDNA</th>
<th>Sequence (5'→3')</th>
<th>Length of product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α₁-isofrom</td>
<td>P1</td>
<td>1,617-1,638</td>
<td>AAGGACGCTTTCTAGAGATGCCT</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>1,863-1,843</td>
<td>TGACCATGATGACCTTAATCC</td>
</tr>
<tr>
<td>α₂-isofrom</td>
<td>P1</td>
<td>2,690-2,710</td>
<td>CACCTACCTTGTAAATAGGC</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>2,953-2,931</td>
<td>ATCCAGATCTTCTTCTATGCC</td>
</tr>
<tr>
<td>α₃-isofrom</td>
<td>P1</td>
<td>1,602-1,621</td>
<td>GACCCCAATGCAACCCGATA</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>1,886-1,866</td>
<td>CATGGACATGAGACCACCGAA</td>
</tr>
<tr>
<td>G3PDH</td>
<td>P1</td>
<td>550-569</td>
<td>ACCACAGTCTATGCCATCAC</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>1,001-982</td>
<td>TCCACCACCTGTGTCGTT</td>
</tr>
</tbody>
</table>
simultaneously as a control (15). The mRNA levels of the $\alpha_1$, $\alpha_2$, $\alpha_3$-subunit isoforms were expressed as relative units compared with the control (G3PDH), respectively.

Specific primers for PCR were chosen with the help of a suite of genetics data base programs (Oligo 5.0) and were synthesized by the Shanghai Institute of Cell Biology, Chinese Academy of Science. Primer sequences are listed in Table 1.

The PCR products quantitatively reflect the amount of initial template DNA only before the reaction reaches the plateau of the amplification curves. Therefore, we must determine the PCR cycles in this reaction system to compare the amount of initial template DNA using RT-PCR methods (16). Figure 1 shows the results found when equivalent RNA aliquots of the $\alpha_3$-subunit were amplified with varying numbers of PCR cycles. The amplification curves of the $\alpha_1$, $\alpha_2$, $\alpha_3$-subunits of the sodium pumps and G3PDH were similar. As shown, the plateau in PCR amplification occurred at about cycle 30, and the amount of PCR product increased exponentially at cycle 25 of this reaction for the sodium pump $\alpha_1$, $\alpha_2$, $\alpha_3$-subunit isoforms and for G3PDH.

**Statistical Analysis**

Values are expressed as mean±SD. The Student’s t-test was performed with Microsoft Excel 5.0 statistical software. P values of less than 0.05 were considered to indicate statistical significance.

**Results**

**Effects of Continued Infusion of Ouabain or Digoxin on SBP**

The SBP of rats began to increase after 2 weeks of oua-

bain administration and increased significantly after 6 weeks compared with the control group, which received normal saline (132.6±9.0 vs. 115.7±8.2 mmHg, p<0.05). No difference in systolic blood pressure was found between the rats administered digoxin and those administered normal saline (Fig. 2).

**Sodium Pump $\alpha$-Subunit Isoform Abundance in Normal SD Rats**

The sodium pump $\alpha_1$, $\alpha_2$, and $\alpha_3$-subunit isoforms are

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**Fig. 1. Amplification curve of PCR**

**Fig. 2. Changes in the SBP of each group during the experiment. Points and bars represent means±SD (n=8 in each group). Statistically significant difference: *p<0.05, **p<0.01 (compared with N group).**

**Fig. 3. Two percent agarose gel of PCR products of sodium pump $\alpha_1$, $\alpha_2$, $\alpha_3$-subunit isoforms and G3PDH. Lane 1: 100 bp DNA ladder; Lanes 2, 6: 247 bp products of RT-PCR from $\alpha_1$-isoform; Lanes 3, 7: 264 bp products of RT-PCR from the $\alpha_2$-isoform; Lanes 4, 8: 285 bp products of RT-PCR from $\alpha_3$-isoform; Lanes 5, 9: 452 bp products of RT-PCR from G3PDH. Products of lanes 2-5 for one tissue and lanes 6-9 for another tissue.**

The $\alpha_1$, $\alpha_2$, and $\alpha_3$-isoform mRNA levels in various tissues were quantified by densitometric scanning and expressed as relative units compared with the control (G3PDH). Columns and bars show means ± SD (n=8 in each group). Statistically significant difference: *p<0.05, **p<0.01 (compared with N group).

Fig. 4. The $\alpha_1$, $\alpha_2$, and $\alpha_3$-isoform mRNA levels in various tissues were quantified by densitometric scanning and expressed as relative units compared with the control (G3PDH). Columns and bars show means ± SD (n=8 in each group). Statistically significant difference: *p<0.05, **p<0.01 (compared with N group).

distributed in a tissue-specific fashion in normal rats. The $\alpha_1$-isoform was found to be ubiquitous in most tissues, and its expression was greater than that of the other two isoforms in the ventricular myocardium, kidney, and adrenal gland. The predominance of the $\alpha_1$-isoform was especially found in the kidney. In the hypothalamus, however, expression of $\alpha_1$-isoform was less than that of the $\alpha_2$- and $\alpha_3$-isoforms. Second, the $\alpha_2$-isoform was expressed abundantly in the ventricular myocardium, aortic smooth muscle, and hypothalamus. The $\alpha_2$-isoform was more abundant than $\alpha_1$ or $\alpha_3$ in aortic smooth muscle. Third, the $\alpha_3$-isoform was expressed abundantly in aortic smooth muscle and the hypothalamus. In addition, we can describe the sodium pump $\alpha$-subunit isofoms abundance in each tissue: $\alpha_1$ and $\alpha_2$ were the predominant isoforms, while much less of the $\alpha_3$ was expressed in the ventricular myocardium; $\alpha_1$ was abundant, while less of $\alpha_2$ and $\alpha_3$ were expressed in the kidney; slightly more $\alpha_1$ than the $\alpha_2$- and $\alpha_3$-isoforms were present in the adrenal gland, while slightly more $\alpha_2$ was present in the aortic smooth muscle; more $\alpha_2$ and $\alpha_3$ than $\alpha_1$ were expressed in the hypothalamus.

Effects of Ouabain and Digoxin on the Expression of the Sodium Pump $\alpha$-Subunit Isoform (Figs. 3, 4)

Expression of the sodium pump $\alpha$-subunit isoform is regulated by either ouabain or digoxin in a tissue-specific fashion. 1) Ventricular myocardium (Fig. 4a): Both ouabain and digoxin stimulated expression of the $\alpha_3$-isoform, whereas the expression of $\alpha_2$ remained unchanged. The $\alpha_1$ expression decreased significantly in the ouabain group and decreased slightly in the digoxin group, respectively. 2) Kidney (Fig. 4b): Digoxin had the same effect on sodium pump $\alpha$-subunit isoform expression as ouabain. The $\alpha_1$ levels increased, while those of $\alpha_2$ and $\alpha_3$ remained unchanged. The $\alpha_1$ expression decreased significantly in the ouabain group and decreased slightly in the digoxin group, respectively. 3) Adrenal gland (Fig. 4c): $\alpha_2$ and $\alpha_3$ levels increased, while those of $\alpha_1$ decreased in the ouabain group. In the digoxin group, $\alpha_1$ and $\alpha_3$ levels increased, and those of $\alpha_2$ remained unchanged. 4) Aortic
smooth muscle (Fig. 4d): Both ouabain and digoxin increased $a_1$ and $a_3$ expression. The $a_2$ levels decreased in the digoxin group but remained unchanged in the ouabain group. 5) Hypothalamus (Fig. 4e): Both ouabain and digoxin, respectively, stimulated $a_1$ expression, which is generally relatively low in normal SD rats, while $a_2$ and $a_3$ levels remained unchanged.

Discussion

It has been demonstrated that ouabain administered intraperitoneally is readily absorbed, and that plasma ouabain levels are not significantly different between intraperitoneal and intravenous groups at 10 min after administration (8, 9). The doses of ouabain infused in our study were determined based on both pharmacokinetic data for ouabain and data obtained from the literature (8, 9). According to ouabain pharmacokinetics in animals, the doses of ouabain infused to the experimental rats in the present study are estimated to increase plasma levels of ouabain to 3–8 times higher (2–5 nmol/l) than physiological levels (the plasma ouabain concentrations in normal animals and human beings are approximately 0.07–0.11 nmol/l), which is similar to the levels present in many pathologic states, including hypertension and congestive heart failure (5). Moreover, based on weight gain, behavior changes, and cardiac rhythms, the pressor effects of ouabain did not appear to be associated with substantial toxicity.

Ouabain has been used clinically primarily as a short-term, intravenous drug for patients with congestive heart failure or atrial arrhythmias who may be unable to demonstrate a pressor response. However, clinical experiences have suggested that the long-term administration of digoxin does not produce hypertension. The present results also show that digoxin does not induce hypertension, indicating that there is a big difference between the effects of ouabain and those of digoxin on blood pressure, although both drugs belong to the digitalis family. Moreover, it has been found that both digoxin and digitoxin can prevent some forms of hypertension (17, 18). The reasons for the difference in effects of ouabain and digoxin on blood pressure are unknown.

According to the traditional view, EO may induce a series of changes in cytobiological effects, thus leading to hypertension by changing the sodium pump configuration and inhibiting its activity; it is possible that this inhibition is the only effect of ouabain on blood pressure, although both drugs belong to the digitalis family. Moreover, it has been found that both digoxin and digitoxin can prevent some forms of hypertension (17, 18). The reasons for the difference in effects of ouabain and digoxin on blood pressure are unknown.

The sodium pump, acting as a “receptor” for cardiac glycosides such as digoxin and ouabain, plays a central role in a variety of physiological processes, including transepithelial ion transport, the regulation of cell
volume, Na\(^+\)-coupled uptake of metabolic substrates (glucose, amino acids), and propagation of the action potential of muscle and nerve (3, 4, 10). It is well known that the \(a_1\)-, \(a_2\)-, and \(a_3\)-isoforms have different affinities for Na\(^+\) and cardiac glycosides (3, 4, 10). According to our new hypothesis, ouabain could affect the activity of sodium pumps by changing either the configuration or the gene expression of sodium pump \(a\)-isoforms. Changes in sodium pump activity might: 1) in turn regulate the gene expression of sodium pump \(a\)-isoforms by changing intracellular Na\(^+\), Ca\(^2+\), and other ion concentrations (10, 19); and 2) interact with the complex neurohumoral regulation, which might then lead to hypertension in many ways, and interact with both sodium pump \(a\)-subunit isoform expression (10) and endogenous ouabain secretion (2).

In summary, there was a great difference between the effects of ouabain and those of digoxin on the blood pressure of rats. Prolonged administration of small doses of ouabain induced hypertension in SD rats, while administration of digoxin did not. Ouabain had a significant effect on the expression of sodium pump \(a_1\)-, \(a_2\)-, and \(a_3\)-subunit isoforms in a tissue-specific fashion, and there were great differences between the effects of ouabain and those of digoxin on \(a\)-mRNA levels. Further determination of the significance of the regulations of sodium pump \(a\)-subunit isoform expression might be great helpful for the insight into the physiological role of endogenous ouabain and the molecular mechanism involved in the pathogenesis of hypertension.

**Abbreviations**

Abbreviations used in this paper: bp, base pair; cDNA, complementary DNA; DNA, deoxyribonucleic acid; dNTP, deoxynucleotide triphosphate; EO, endogenous ouabain; G3PDH, glyceraldehyde-3-phosphate-dehydrogenase; mRNA, message RNA; NS, normal saline; PCR, polymerase chain reaction; RNA, ribonucleic acid; RT, reverse transcription; SBP, systolic blood pressure; SD, Sprague-Dawley.

**Acknowledgements**

The authors would like to thank Dr. Yu-Kang Yuan and Dr. Hui-Xun Ren of the Department of Immunology, Xi'an Medical University, for their invaluable support and advice in conducting this work.

**References**