Effects of ouabain and digoxin on gene expression of sodium pump α -subunit isoforms in rat myocardium

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Keywords: ouabain， digoxin， sodium pump， myocardium， rat

Objective To compare the effects of ouabain and digoxin on the gene expression of sodium pump α-subunit isoforms in the myocardium of rats.

Methods Normal Sprague-Dawley (SD) rats were injected with ouabain (20 μg • kg^{-1} • d^{-1},i.p.), digoxin (32 μg •kg^{-1} •d^{-1},i.p.) and normal saline (NS) once a day, respectively, and indirect systolic blood pressure was recorded once a week. Six weeks later, all of the rats were killed, and sodium pump α_1-, α_2-, and α_3-subunit mRNA levels in the myocardium were detected with the reverse transcription polymerase chain reaction (RT-PCR) method.

Results The systolic blood pressure of the rats infused with ouabain increased significantly at the end of week 6 (132.6±9.0mm Hg vs 115.7±8.2 mm Hg, $p<0.01$), while no difference in blood pressure was found between the digoxin group and the NS group. The expression of sodium pump α-subunit isoforms in the ventricular myocardium was regulated by either ouabain or digoxin. Both ouabain and digoxin stimulated expression of the α_3-isoform, whereas $\alpha_2$was unchanged in those two groups. α_1-isoform expression decreased in the ouabain group and was unchanged in the digoxin group.

Conclusions These results suggest that both ouabain and digoxin could regulate sodium pump α-subunit isoform expression, which might be related to the physiological roles of endogenous ouabain and might be responsible for the difference in the pharmacological and toxicological effects of ouabain and digoxin, including their effects on blood pressure.

Accumulating evidence indicates that endogenous ligands of digitalis which has recently been described 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 bady.[2,3] Many studies have showed that EO contents in both hypertensive patients and hypertensive animals are much higher than those of normal rats, suggesting that higher EO might be involved in the development of hypertension.[2-5] However, the mechanism by which ouabain induces hypertension is not completely clear. It has been suggested that EO induces a series of cytobiologcal changes and leads to hypertension by changing sodium pump (Na^+, K^+-ATPase) configuration and inhibiting its activity, and this has been possibly seen as the only effect of EO on the sodium pump.[2-5]

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 the ouabain binding sites and possesses specialized functions. The β-subunit is a glycoprotein that is essential to normal function and the assembly of the enzyme.[3,6] These isoforms are expressed in a tissue- and cell-specific fashion, and are controlled by developmental and hormonal regulatory influences.[3,6] Also, this gene expression is not well understood. This study was designed to investigate the effect of ouabain on sodium pump α

-subunit gene expression is not well understood. This study was designed to investigate the effect of ouabain on sodium pump α-subunit gene expression in myocardium compared with that of digoxin.

METHODS

Ouabain and digoxin infusion and blood pressure measurement

Twenty-four healthy adult male Sprague-Dawley (SD) rats (6-10 weeks old) weighing 200-250g 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 one week, the body weight and systolic blood pressure of the rats were determined to document normotension. They were divided randomly into 3 groups: the ouabain group (O group, n=8), the digoxin group (D group, n=8) and the normal saline group (N group, n=8). Animals in these 3 groups were given, respectively, ouabain (Sigma Chemical Co., 20 μg • kg^{-1} • d^{-1}), [10,11] digoxin (Sigma Chemical Co., 32 μg • kg^{-1} • d^{-1}) and normal saline (1 ml • kg^{-1} • d^{-1}) intraperitoneally daily. Feeding and living conditions were the same among these groups throughout the study. Indirect systolic blood pressure (SBP) was recorded with 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 rats were fasted overnight and killed by decapitation the following morning. The left ventricular myocardium was collected to determine the mRNA level of the sodium pump α-subunit isoform using reverse transcription polymerase chain reaction (RT-PCR) techniques.

RT-PCR

Total RNA was extracted from the myocardium as previously described[12] and digested with 5 unit RNase free DNAse (Boehringer Mannheim, Germany). Total RNA 1 μg was used to synthesize the first strand cDNA. RNA samples were heated to 95℃ for 4 min, chilled on ice, and reverse transcriptase, 400 μ mol • L^{-1} of each deoxynucleotide triphosphate (dNTP: dATP, dTTP, dCTP, and dCTP), 50 mmol/L Tris-HCl (pH 8.3), 75 mmol •L^{-1}KCL, and 3 mmol •L^{-1} $MgCl_2$. The reaction was terminated by heating to 65℃ for 10 min. Equal volumes of resultant products were amplified in a final volume of 50 μl containing the following:200 μ mol • L^{-1} dNTP, 5 pmol each of forward and reverse primers, 10 mmol • L^{-1} Tris-HCl (pH 8.8), 50 mmol • L^{-1} KCl, 1.5mmol • $L^{-1}$$MgCl_2$, 0.1% Triton X-100, and 2.5 unit Taq polymerase (Promega, Madison, USA). Samples were denatured at 94℃ for 2 min and cycled 25 times through the following steps:1 min at 94℃, 1 min at 52℃, and 2 min at 68℃. The final cycle was extended for 7 min at 68℃. PCR products were electrophoresed on 2% agarose gels (Fig. 1) and quantitated by scanning densitometry (Gelworks ID Intermediate 3.01). RT-PCR of the glyceraldehydes-3-phosphate-dehydrogenase (G3PDH) gene was performed simultaneously as a control.[13] The α_1-, α_2-, α_3-subunit isoform mRNA levels were respectively expressed as relative units compared with the control (G3PDH).[13]

Specific primers for PCR were chosen with the help of a genetics database program (Oligo 5.0)and were synthesized by the Shanghai Institute of Cell Biology, Chinese Academy of Science. Primer sepuences are listed in the Table.

Table. Primer sequences for amplifying sodium pump α_1-、α_2-、α_3-subunit isoform and G3PDH

Primer	Position of bp in cDNA	Sequence(5'→3')	Length of product(bp)

specificity				
α_1-isoform	P1	1617-1638	AAGGACGCCTTTCAGAATGCCT	247
	P2	1863-1843	TGACCATGATGACCTTAATCC	
α_2- isoform	P1	2690-2710	CACCTACTTTGTAATACTGGC	264
	P2	2953-2931	ATCAGGATCTTGTTCTTCAGCC	
α_3- isoform	P1	1602-1621	GACCCCAATGACAACCGATA	285
	P2	1886-1866	CATGGACATGAGACCCACGAA	
G3PDH	P1	550-569	ACCACAGTCCATGCCATCAC	452
	P2	1001-982	TCCACCACCCTGTTGCTGTT	

PCR product quantitatively reflects the amount of initial template DNA only before the reaction reaches the plateau of amplification curves. We must therefore determine the number of PCR cycles used in this reaction system so that the amounts of initial template DNA can be compared with each other using RT-PCR.[14] Fig. 1 shows the results found when equivalent RNA aliquots of the α_3 subunit were amplified with varying numbers of PCR cycles. The amplification curves of the α_1-, α_2-, α_3-subunits of the sodium pump and G3PDH were similar to it. As shown (Fig. 2), the plateau in PCR amplification occurred at about cycle 30, and the amount of PCR product increases exponentially at cycle 25of this reaction for sodium pump α_1-, α_2-, α_3-subunit isoforms and for G3PDH.

Fig.2.

Statistical analysis

Values are expressed as $\bar{x} \pm s$. The Student's t test was performed with Microsoft Excel 5.0.

RESULTS

Effects of continued infusion of ouabain or digoxin on SBP

The systolic blood pressure of the rats began to increase after two weeks of ouabain administration and increased significantly after six weeks compared with that of the control group, which received normal saline (132.6±9.0mm Hg vs 115.7±8.2 mm Hg, $p<0.01$). No difference in systolic blood pressure was found between the rats administrated with digoxin and those with normal saline (Fig. 3).

Fig.3.

Effects of ouabain and digoxin on the expression of sodium pump α -subunit isoforms

The results have showed that the sodium pump α_1- and α_2-isoforms were the predominant isoforms, and α_3 was much less expressed in the ventricular myocardium of normal rats. The expression of sodium pump α -subunit isoforms in the ventricular myocardium was regulated by either ouabain or digoxin. Both ouabain and digoxin stimulated the expression of α_3-isoform, whereas α_2 was unchanged in those two groups. α_1-isoform expression decreased in the ouabain group and was unchanged in the digoxin group (Fig. 4)

Fig. 4

DISCUSSION

It has been demonstrated that ouabain administered intraperitoneally was readily absorbed, and plasma ouabain levels were not significantly different between the intraperitoneal and intravenous groups at 10 minutes after administration.[10,11] The doses of ouabain infused in our study were determined based on the pharmacokinetic data on ouabain and referred to some references, [10,11]

The doses of ouabain infused in our study were determined based on the pharmacokinetic data on ouabain and referred to some references,[10,11] and the dose of digoxin was determined according to preliminary examination and previous research.[15] According to ouabain pharmacokinetics in animals, the doses of ouabain infused to the experimental rats were estimated to increase the plasma levels of ouabain to 3-8 times higher (2-5nmol/L) than that of the physiological level (the plasma ouabain concentration in normal animals and humans is about 0.7-1.1nmol/L), similar to those in many pathologic states, such as hypertension and congestive heart failure.[4] Moreover, based on weight gain, rats behavior and cardiac rhythm, 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 in patients with congestive heart failure or atrial arrhythmias, which may not demonstrate the pressor response. However, clinical experiences have suggested that the long-term administration of digoxin did not produce hypertension. Also, the present results showed that digoxin could not induce hypertension, indicating that there is a big difference between the effects of ouabain and digoxin on blood pressure, although both of them belong to the digitalis family. Moreover, it has been found that both digoxin and digitoxin which is very similar to digoxin in both molecular structure and biological activity, can decrease hypertension significantly. [16,17] The reasons for the differences between the effects of ouabain and digoxin on blood pressure are unknown.

It has been thought that EO might induce a series of cytobiological effects and lead to hypertension by changing sodium pump configuration and inhibiting its activity, and this has been seen as possibly the only effect of ouabain on the sodium pump.[3] But there are two questions regarding this view. First, digoxin is another member of the digitalis family, and it also causes cardiac and vascular smooth muscle to contract by inhibiting the activity of the sodium pump like ouabain, but clinical experience and the results of this study suggested that digoxin could not induce hypertension during long periods of administration, and it could even prevent the development of hypertension.[15,16] Second, the EO levels in physiological and even in some diseased conditions are not as high as those used in most studies in vitro, indicating that unless there are ouabain-sensitive forms of sodium pump α -subunits (α_2 and α_3), the lower concentrations of circulating ouabain might not be adequate to inhibit the enzyme and to play an important role in vascular control under physiological and pathophysiological conditions.[4] Based on our previous studies and related research papers, we put forward the hypothesis that ouabain induces some biological changes in the related tissues and increases blood pressure not only by changing sodium pump configuration and inhibiting its activity, but also, at least in part, by causing abnormal expressions of the sodium pump α subunit isoform genes.

The sodium pump has been reported to play a central role in a variety of physiological processes, including transepithelial ion transport, regulation of cell volume, Na^+-coupled uptake of metabolic substrates (glucose, amino acids), and the propagation of action potentials of muscles and nerves.[3,6]It is well known that the α_1-, α_2-,and α_3-isoforms,have different affinities for Na^+ and cardiac glycosides. [3,6]According to our new hypothesis, ouabain could affect sodium pump activity by changing either the configuration or the gene expression of sodium pump α isoforms. Changes in sodium pump activity might in turn regulate the gene expression of sodium pump a isoforms by changing intracellular Na^+, Ca^{2+} and other ion' concentrations; [6,18]interact with the complex neurohumoral regulation, which might consequently lead to hypertension and interact with both sodium pump α -subunit isoform expression[6] and endogenous ouabain secretion.[2]

In summary, there was a great difference between the effects of ouabain and digoxin on the blood pressure of rats. Prolonged ouabain administration of small doses induced hypertension in the SD rats but idgoxin could not. Also, there were great differences between the effects of ouabain and digoxin on the expression of sodium pump α -subunit isoforms, which might be related to the physiological roles of EO and might be responsible for the differences between the effects of ouabain and digoxin on blood pressure. These results have provided us experimental evidence on the relationship among ouabain sodium pump and hypertension, and might be very us experimental evidence on the relationship among ouabain, sodium, pump and hypertension, and might be very useful for studying the mechanism of hypertension and the application of digitalis clinically.

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