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Different Distribution of the α_2 Na^+, K^+-ATPase Isoform between Rat Atria and Ventricles

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Rat ventricles respond with a biphasic positive inotropic effect to ouabain, low-dose and high-dose effects but rat atria with only a monophasic high dose effect. In an effect to understand the difference in response to ouabain of two tissues between rat atria and ventricles the levels of the α_2-isoform of the Na^+ , K^+-ATPase which has higher affinity for ouabain than the α_1-isoform were determined by a [^{3}H]ouabain binding assay. The yield of protein per gram wet weight was about 47 mg for atria and 100 mg for ventricles. The K_d values of ouabain for the high-affinity ouabain binding site (α_2-isoform) were nearly the same (230 nM) in the atria and ventricles. However, the numbers of the α_2-isoform (B_{max}) per mg protein were approximately half in the atria. When the binding data were expressed in unit per gram tissue wet weight, the numbers of α_2-isoform in the atria was about 25% of that in the ventricles. These results demonstrate that the α_2-isoform of the Na^+, K^+-ATPase in the rat atria could be detected by [^{3}H]ouabain binding assay and the levels of this isoform are too low to show the low-dose effect of ouabain.

Key word: Na^+, K^+-ATPase, α_2-isoform, [^{3}H]ouabain binding, Atrium, Ventricle

INTRODUCTION

Na^+, K^+-ATPase, an important membrane enzyme responsible for translocating Na^+ and K^+ across the plasma membrane of mammalian cells, is composed of two subunits, a catalytic α-subunit and a glycosylated β-subunit (Akera and Brody, 1978; Schwartz *et al.*, 1975; Sweadner, 1989). The cardiac glycoside ouabain binds to the extracellular surface of the α-subunit and specifically inhibits this enzyme activity (Schwartz *et al.*, 1975; Wallick *et al.*, 1979; Shull *et al.*, 1985). However, there are species and organ differences in the sensitivity of Na^+, K^+-ATPase to ouabain (Ku *et al.*, 1976; Tobin and Brody, 1972). The rat heart Na^+, K^+-ATPase is 10,000 times more resistant to ouabain than the human heart Na^+, K^+-ATPase (Erdmann *et al.*, 1976). Furthermore, in the relatively insensitive rat heart, the ventricles exhibit two classes of [^{3}H] ouabain binding sites with 100−200 folds different affinity for ouabain (Erdmann *et al.*, 1980) and the biphasic low- and high-dose positive inotropic responses to ouabain (Adams *et al.*, 1982; Grupp *et al.*, 1984), whereas the atria show only monophasic high-dose effect of ouabain (Grupp *et al.*, 1984; 1986).

A number of observations of the heterogeneity of Na^+, K^+-ATPase in different tissues led to the discovery of Na^+, K^+-ATPase isoforms. The α-subunit exists in three isoforms with different affinity for cardiac glycosides; α_1 (low affinity), α_2 (high affinity), and α_3 (very high affinty) (Berrebi- Bertrand *et al.*, 1990; Foly and Linnoila, 1993; O'Brien *et al.*, 1994; Sweadner, 1979). These three isoforms also exhibit differential tissue distribution. The α_1- isoform is found in all cells, whereas the α_2 is present predominantly in skeletal muscle and α_3 in neural tissue (Emanuel *et al.*, 1987; Lytton *et al.*, 1985). Young and Lingrel (1987) demonstrated that both α_1 and α_2 mRNAs were expressed in the rat ventricles and atria but the relative proportion of the α_2 mRNAs to the α_1 mRNA was significantly lesser in the atria than in the ventircles, indicating that the difference in the inotropic response to ouabain between the rat ventricles and atria is due to the relative abundance of the α_1 and α_2 mRNAs.

Thus, identification and the relative abundance of the α_2-isoform in the rat atria have been obtained with mRNA probes (Young and Lingrel, 1987; Orlowski and Lingrel, 1988) and with specific antibodies (Charlemagene *et al.*, 1987; Lucchesi and Sweadner, 1991) but the level of this isoform in the

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rat atria has not been quantitated with [^{3}H]ouabain displacement assay, which has been used to detect the α_2-isoform in the rat ventricles (Lee *et al.*, 1983; 1993; Whitmer *et al.*, 1986). In the present study we quantitated the α_2- isoform of the rat atria with [^{3}H] ouabain displacement assay.

MATERIALS AND METHODS

Materials

[^{3}H]Ouabain (19.5 Ci/mmol) was purchased from Dupont-New England Nuclear, Boston, MA, 02118 U. S.A. Ethylenediamine tetraacetic acid (EDTA), ouabain octahydrate were obtained from Sigma Chemical Co., St. Louis, Mo, U.S.A. All other reagents were of reagent grade.

Preparation of atrial and ventricular homogenate

Male Sprague-Dawley Rat (Laboratory supplies, Indianapolis, Indiana) weighing 250~300 g was decapitated and heart was rapidly removed and then divided into atria and ventricles in ice-cold 10 mM Tris·Cl and 1 mM EDTA-imidazole (pH 7.4) medium (medium A). The pooled atrial or ventricular tissues were minced with scissors and homogenized twice for 5 sec at one-half maxium speed in 10 volumes of medium A with a polytron PT-20. The homogenates were centrifuged at 73,400×g for 30 min and the pellet was resuspended in 2 ml of medium A using motor driven teflon pestle (5 passes, 5 times). Protein content was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Measurement of [^{3}H]ouabain binding

Binding of [^{3}H]ouabain was carried out in medium containing 50 mM Tris·Cl (pH 7.4), 5 mM $MgCl_2$, 5 mM Tris·Pi, and 100 nM [^{3}H]ouabain. After incubation of homogenates (200 μg/ml) for 90 minutes at 37°C with 100 nM [^{3}H]ouabain, the reaction mixture was rapidly filtered through 0.45 μm methyl-cellulose filter (Gelman). The filters were washed three times with 5 ml of ice-cold 100 mM KCl and incubated for 10 hours in scintillation fluid prior to determination of radioactivity. Nonspecific ouabain binding observed in the presence of 5.5 mM unlabeled ouabain was subtracted from values observed in its absence to calculate specific [^{3}H]ouabain binding.

Estimation of the ouabain binding site concentration (B_{max}) and affinity (K_D)

The affinity (K_D) and concentration of maximum binding site (B_{max}) to [^{3}H]ouabain were estimated from displacement assay described by Akera and Cheng (1977). For displacement assay of [^{3}H]ouabain binding, protein were incubated at 37°C for 90 min in the presence of various concentrations of unlabeled ouabain with 100 nM [^{3}H]ouabain. The apparent affinity (K_D) and B_{max} were calculated by the following equations;

$$K_D = C_{0.5} - a$$

$$B_{max} = Be \cdot C_{0.5}/a$$

where $C_{0.5}$ is the concentration of unlabeled ouabain which caused a 50% reduction of the labeled ouabain bound at equilibrium, a is the [^{3}H]ouabain concentration (100 nM), and Be is the equilibrium binding of [^{3}H]ouabain in the absence of unlabeled ouabain.

Statistics

All values reported represent the mean±S.E.M. Statistical analysis was performed with Student's *t*-test, and the level for significance was taken as $p<0.05$.

RESULTS AND DISCUSSIONS

Organ weight and protein yield

The atrium was paired with the ventricle to prepare homogenate and studied concomitantly. The atria and ventricular weight measured after sacrifice were about 70 mg and 980 mg, respectively and the protein yield in milligrams of protein per gram of wet weight of atrium was two-fold less than that of ventricle. Consequently, the yield of protein expressed in terms of milligrams of protein per atria was 28-fold less than that of ventricle (Table I).

[^{3}H]Ouabain equilibrium binding

In rat ventricular myocardium, there are two different ouabain binding sites. One site, characterized by a low affinity for ouabain, represents a large proportion of the total number of sites, as detected by sodium-dependent phosphorylation. The other site with a higher affinity which represents a small proportion of the total number of sites has been detected by the [^{3}H]ouabain binding assay (Erdmann *et al.*, 1980; Sharma and Banergee, 1978; Adams *et al.*, 1982). Therefore, we carried out [^{3}H]ouabain equilibrium binding assay to measure a high affinity site for oua-

Table I. Atrial and ventricular weights and protein contents in rats

	Weight (g)	protein	
		mg/g wet wt.	mg/organ
Atrium	0.072±0.008	46.5±2.2	3.5±0.2
Ventricle	0.980±0.030	99.5±4.4	97.5±2.7

Values represent the mean±S.E.M. obtained from 10 rats.

Table II. [^{3}H]Ouabain equilibrium binding to atrial and ventricular homogenate

[^{3}H]ouabain equilibrium binding (p mol/mg protein)	
Atrium	Ventricle
0.39±0.03	0.76±0.10

[^{3}H]Ouabain binding was carried out in the presence of 50 mM Tris·Cl (pH 7.4), 5 mM $MgCl_2$, 5 mM Tris·Pi, 100 nM [^{3}H]ouabain, and 200 μg of homogenate protein for 90 minutes at 37°C as described in Methods.
Values are the mean±S.E.M. from 10 atria and ventricles.

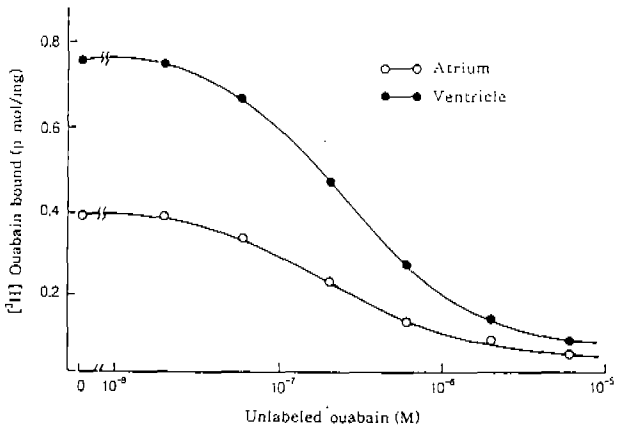

Fig. 1. A typical inhibition curve of [^{3}H]ouabain binding to homogenate by unlabeled ouabain. The binding of a fixed concentration of [^{3}H]ouabain (100 nM) was assayed in the presence of various concentrations of the unlabeled ouabain. Nonspecific ouabain binding observed in the presence of 5.5 mM unlabeled ouabain was subtracted from values observed in its absence to calculate specific [^{3}H]ouabain binding.

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As shown in Table II, the equilibrium binding of ouabain in the presence of 100 nM [^{3}H]ouabain was approximately 0.4 and 0.8 pmol per mg protein in atria and ventricles, respectively. This result further supports that a high affinity ouabain binding site in rat ventricle can be easily detected by the [^{3}H]ouabain binding assay and indicates that rat atrium also contains the high affinty site for ouabain but there are differences in the distribution of this site between atrium and ventricle.

Displacement assay of [^{3}H]Ouabain binding

The Na^+, K^+-ATPase has three α isoforms with different affinity for cardiac glycosides: α_1, α_2, and α_3 with the apparent K_d value of 1~20 nM, 40~500 nM, and 30~100 μM, respectively (Berrebi-Bertrand *et al.*, 1990; Blanco *et al.*, 1993; O'Brien *et al.*, 1994). In order to identify the isoform of Na^+, K^+-ATPase which corresponds to the [^{3}H]ouabain binding site detected in rat atria and ventricles, the apparent K_d

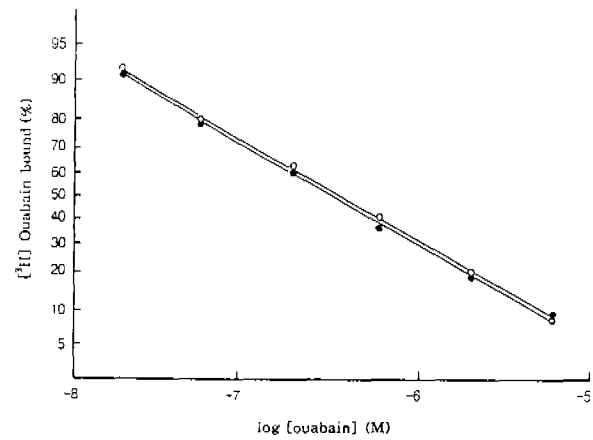

Fig. 2. Probit plot of data obtained from [^{3}H]ouabain displacement assay. Each point (mean of ten determinations) represents the percent decrease of specific [^{3}H]ouabain bound at equilibrium displaced by increasing concentrations of unlabeled ouabain. 100 nM [^{3}H]ouabain was used in this assay. See Methods for details of binding assay.

Table III. K_d value and ouabain binding site concentration in cardiac homogenate

	K_D (nM)	B_{max} (p mol/mg protein)
Atrium	234±25.2	1.3±0.11
Venticle	221±20.7	2.4±0.17*

K_D and B_{max} were calculated by the following equation: $K_D=C_{0.5}-a$, $B_{max}=Be\ C_{0.5}/a$, where $C_{0.5}$ is the concentration of unlabeled ouabain which caused a 50% reduction of the labeled ouabain bound at equilibrium, a is the [^{3}H] ouabain concentration (100 nM), and Be is equilibrium binding of [^{3}H] ouabain in the absence of unlabeled ouabain. $C_{0.5}$ was 334± 25.2 nM for ventricle.
Values represent the mean±S.E.M. from ten atria and ventricles.
*: Significantly different ($p < 0.01$) from corresponding value of atrium

values and the numbers of ouabain binding sites were estimated by a [^{3}H]ouabain displacement assay. When the binding of fixed concentration of ouabain (100 nM) was assayed in the presence of various concentrations of the unlabeled ouabain, it was dose-dependently inhibited by unlabeled ouabain (6×10^{-8}~ 6×10^{-6} M) (Fig. 1). In Fig. 2, the binding of [^{3}H]ouabain in the presence of unlabeled ouabain is expressed as a percentage of that measured in the absence of unlabeled ouabain (probit scale) and plotted against the unlabeled ouabain concentration (in logarithmic scale). From this figure the concentration of ouabain which half-maximally inhibits [^{3}H]ouabain binding was the same ($C_{0.5}\cong350$ nM) for atrium and ventricle. Therefore, the apparent K_d values of 220~ 230 nM, calculated from the equation shown in the Method, were not different in the two organs, indicating that the isoform of Na^+, K^+-ATPase detected

Table VI. [^{3}H]Ouabain binding to rat atrium and ventricle expressed in different units

Parameters	n	Atrium	Ventricle
Ouabain binding (p mol)			
per mg protein	10	1.3±0.11	2.4±0.17
per wet tissue	10	60.5±4.8	238.8±15.4
per organ	10	4.6±0.3	234 ±10.6
No. of high affinity site			
Per g wet tissue		3.64×10^{13}	14.38×10^{13}
per organ		0.28×10^{13}	14.09×10^{13}

n=the number of organs used

by [^{3}H]ouabain binding in the atrium and ventricle is the α_2 isoform. However the binding site concentration (B_{max}) in the atrium was markedly lower than that of ventricle (Table III).

Grupp *et al.* (1981) demonstrate that ouabain have two types of positive inotropic action; these are low and high dose response in rat ventricle but in the atria, high dose response is only showed. Also, Adams *et al.* (1982) suggest that two types of ouabain binding site coexist in the rat ventricles; though, in the atria, high affinity site does exists very little or not. In that case, it is difficult to detect the positive inotropic effects of the inhibition of ouabain high affinity sites. Also, our data indicates that it is hard to detect the effect of ouabain high affinity site in the atria on account of the low number of α_2 isoform of Na^+, K^+-ATPase.

The level of the α_2-isoform in the atria and ventricles

The level of the α_2-isoform was calculated from the organ weight, protein yield and the ouabain binding site concentration (B_{max}). As shown in Table IV, the binding site concentration per gram of wet tissue of atrium was markedly lower (25%) than that ventricle. If one molecule of ouabain binds to one molecule of the α_2-isoform, atrium and ventricle contain 3.6×10^{13} and 14.4×10^{13} molecules per gram of wet tissue, respectively. Thus, the ratio of the atrial to ventricular α_2-isoform is roughly 1 : 4.

Since the α_2-isoform of Na^+, K^+-ATPase has a greater affinity for ouabain than the α_1-isoform, the greater distribution of the more sensitive α_2-isoform is directly correlated with the greater low dose positive inotropic response to ouabain. In rat ventricle, it has been known that the maximal high affinity response to ouabain is about 30% of the total response (Akera *et al.*, 1973; Adams *et al.*, 1982). Accordingly, it is almost impossible to detect the low dose inotropic response by ouabain occupation to the α_2-isoform in the rat atrium which contain 25% of ventricular α_2-isoform per gram wet tissue. In conclusion, we confirmed the existence of the α_2-isoform in the rat atria with [^{3}H]ouabain binding assay. However, the level of α_2-isoform is significantly lower in the atria than in the ventricle, which account for the no high affinity (low dose) response to ouabain in the rat atria.

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