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SPECIES DIFFERENCE IN TEMPERATURE DEPENDENCE OF CARDIAC ($Na^+ + K^+$)-ATPase ACTIVITY

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Abstract—Cardiac ($Na^+ + K^+$)-ATPase from several species of animals were comparatively studied with respect to their molecular form and temperature dependence. The molecular weight of the catalytic subunit varied a little among species, but the difference did not correlate with the sensitivity of the enzyme to ouabain inhibition. Analysis of Arrhenius plots of the activities of the enzymes showed that enzymes showing break points of 24–25° were relatively insensitive to ouabain inhibition whereas those enzymes with break points of 29–31° were much more sensitive to the glycoside. This suggests that there is a difference in the interaction of cardiac ($Na^+ + K^+$)-ATPase with lipids between the ouabain-sensitive and -insensitive animals.

($Na^+ + K^+$)-ATPase (EC 3.6.1.3), an enzymatic activity involved in the active translocation of Na^+ and K^+ across cell membranes, is specifically inhibited by ouabain or other cardiac glycosides. In the heart, the enzyme is thought to be a putative receptor for cardiac glycosides and a mediator for their positive inotropic action [1–3]. This idea is supported by the fact that sensitivity of cardiac ($Na^+ + K^+$)-ATPase to ouabain inhibition varies considerably among different species, and the difference parallels the sensitivity of the heart to the positive inotropic effect of the drug. The clarification of the determinants of the species difference is important, because it will undoubtedly contribute to a better understanding of the nature of the interaction of the enzyme with cardiac glycosides. However, the biochemical basis for the species difference in sensitivity of cardiac ($Na^+ + K^+$)-ATPase to ouabain inhibition is not fully clarified. In this paper, we examine the effect of temperature on the activities of cardiac ($Na^+ + K^+$)-ATPase from various species in order to study the possible role of lipids around the enzyme protein in ouabain sensitivity, since the discontinuity in Arrhenius plots of the enzyme activity is attributed to the interaction of the enzyme protein with a tightly associated lipid annulus [4–6]. The molecular form of cardiac ($Na^+ + K^+$)-ATPases was also examined, in view of the report [7] that there are two molecular forms [termed $\alpha(+)$ and α] of ($Na^+ + K^+$)-ATPase in brain, one more sensitive to ouabain inhibition than the other.

MATERIALS AND METHODS

[γ-^{32}P]ATP was obtained from the New England Nuclear Corp. Vanadium-free Tris-ATP was purchased from the Sigma Chemical Co. Ouabain was from E. Merk A.G. All other chemicals used were of the highest purity commercially available. Male mongrel dogs (7–10 kg), male rabbits (2–2.5 kg), male guinea pigs (350–450 g), male Sprague–Dawley rats (150–250 g) and male ddY mice (20–30 g) were purchased from commercial suppliers. Bovine hearts were obtained fresh from a slaughterhouse. Their ventricular muscles were frozen and stored until use. Cardiac ($Na^+ + K^+$)-ATPases were partially purified by gentle extraction with sodium dodecyle sulfate (SDS) as described previously [8]. This procedure gave 5- to 10-fold purification with about 5% protein recovery from the microsomes in all species examined.

The procedure for assay of ($Na^+ + K^+$)-ATPase activity was essentially the same as that reported previously [9]. The reaction time was varied between 2 and 25 min, depending on the incubation temperature which was controlled with a Coolnics Circulator. In the ouabain sensitivity experiment, the reaction was carried out for 30 min. The standard medium contained 25 mM imidazole-buffer (pH 7.25), 3 mM $MgCl_2$, 20 mM KCl, 140 mM NaCl and 3 mM ATP. Mg^{2+}-ATPase activity was measured in the absence of Na^+ and K^+. The amount of enzyme protein in each assay was adjusted to allow hydrolysis of less than 10% of the total ATP. The hydrolytic activity was linear with respect to protein concentration and time at all temperatures under the conditions. Activity is shown as μmoles P_i/mg protein/min. Protein was determined by the method of Lowry *et al.* [10].

Phosphorylation of the enzyme by [γ-^{32}P]ATP and SDS-polyacrylamide gel electrophoresis (PAGE) was carried out as described previously [7, 8]. Only the area of the gel around the phosphorylated intermediate was photographed since Na^+-stimulated and K^+-inhibited phosphorylation occurred only at the band on the gel.

For analysis of temperature dependence of the enzyme, the log specific activity of each enzyme was plotted against the reciprocal of the absolute temperature of the incubation medium. For calculation of the exact temperature at discontinuity

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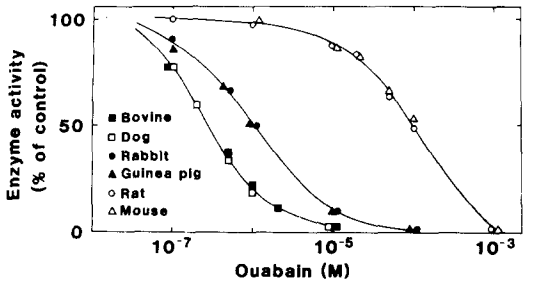

Fig. 1. Dose–response curves of cardiac $(Na^+ + K^+)$-ATPase activities versus ouabain concentration. The specific activities (μmoles P_i/mg protein/min) of the enzymes used here were: 0.90 ± 0.11 (bovine), 1.87 ± 0.02 (dog), 1.86 ± 0.41 (rabbit), 1.78 ± 0.29 (guinea pig), 1.35 ± 0.04 (rat), and 2.77 ± 0.52 (mouse) (N = 2–5). Each point represents the means of at least three separate experiments.

(T_d), separate linear regressions were calculated by the least mean squares method as reported by Rangaraj and Kalant [11]. The intersection of the calculated regression lines for each enzyme was used to define the T_d, while the slopes of the lines were used to calculate the energy of activation above and below T_d [12].

RESULTS

Figure 1 shows the effect of ouabain on the activities of the partially purified $(Na^+ + K^+)$-ATPases from various animal species. The enzymes from bovine and dog were highly sensitive to ouabain inhibition and those from rabbit and guinea pig were moderately sensitive. On the other hand, rat and mouse enzymes were relatively insensitive to ouabain inhibition. In this study, the highly sensitive and moderately sensitive enzymes are referred to as sensitive, and the relatively insensitive enzymes are referred to as insensitive. About a 100- to 350-fold difference was observed in the concentration of ouabain needed to produce 50% inhibition of cardiac $(Na^+ + K^+)$-ATPase in the sensitive and insensitive animals under the assay conditions.

The enzymes were phosphorylated by [γ-^{32}P]-ATP in the presence of Mg^{2+} and Na^+ and then analyzed by SDS-PAGE (Fig. 2). The autoradiography of the gel shows a single band at M_r = 95,000 except for dog enzyme in which the phosphorylated intermediate is observed as the doublet bands.

The typical temperature dependence of cardiac $(Na^+ + K^+)$-ATPase activity is shown as Arrhenius plots in Fig. 3. A discontinuity in the plots was observed in all enzyme preparations. The T_d values and activation energies above and below T_d of cardiac $(Na^+ + K^+)$-ATPases from various animal species are summarized in Table 1. The T_d values for the enzymes of the ouabain-sensitive species (bovine, dog, guinea pig and rabbit) were 29–31°, while those of the insensitive species (rat and mouse) were 24–25°. The difference in T_d between the sensitive and insensitive enzymes was significant statistically. With respect to the activation energy, there were some variations among the species, but they did not appear to be related to the species difference in ouabain sensitivity.

DISCUSSION

The insensitivity of rat cardiac $(Na^+ + K^+)$-ATPase to ouabain inhibition was explained originally by the formation of an unstable complex between the enzyme and ouabain [1]. Since then, some biochemical explanations for the differences in ouabain sensitivity of $(Na^+ + K^+)$-ATPase in various tissues have been proposed. Periyasamy *et al.* [13] reported that the determinant of the insensitivity of rat kidney $(Na^+ + K^+)$-ATPase is contained within the primary structure of the enzyme protein. Sweadner [7] demonstrated that there are two molecular forms of the enzyme in the brain, that is, $\alpha(+)$ and α forms; they differ in affinity for strophanthidin. This finding suggests that the difference in the ouabain sensitivities of cardiac enzymes may depend on whether they contain the $\alpha(+)$ or the α form of the catalytic subunit. There are also several reports suggesting a role for lipids in the interaction of $(Na^+ + K^+)$-ATPase with ouabain. Taniguchi and Iida [14] have reported that phospholipids play a role in the binding of ouabain to $(Na^+ + K^+)$-ATPase. Akera *et al.* [15] have suggested that the low sensitivity of rat heart to cardiac glycosides may result from the lack of a lipid barrier to regulate the release of the glycosides from their binding sites. Charnock *et al.* [16] suggested that membrane lipids might be involved in the sensitivity. This is further supported

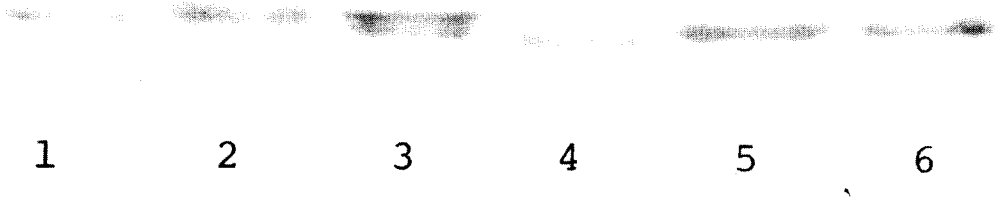

Fig. 2. Autoradiograph of SDS-PAGE of cardiac $(Na^+ + K^+)$-ATPases. The partially purified enzymes were incubated by [γ-^{32}P]ATP and subjected to SDS-PAGE. The concentration of acrylamide was 5%. The lanes from left to right are: (1) rat, (2) mouse, (3) dog, (4) rabbit, (5) guinea pig, and (6) bovine.

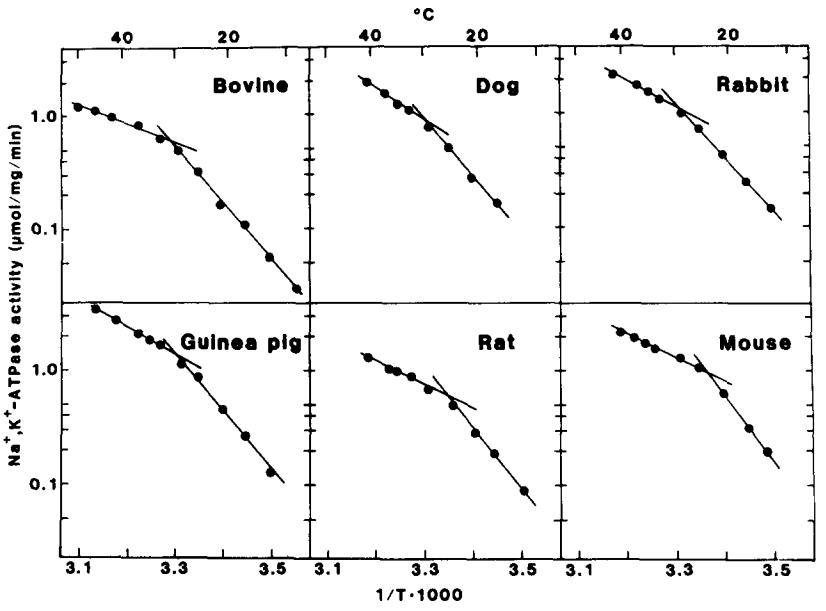

Fig. 3. Arrhenius plots of (Na^+ + K^+)-ATPase activities of the partially purified enzymes from various animals. A typical experiment of Arrhenius plots for each enzyme is shown. Temperature, in degrees centigrade, is shown on the horizontal scale at the top of the panel.

by a recent experiment using a reconstitution system [17], though Hegyvary *et al.* [18] question this idea.

In this study, we first examined the molecular form of cardiac (Na^+ + K^+)-ATPases. SDS-PAGE of these enzymes showed that two molecular forms [corresponding to $\alpha(+)$ and α forms] were present in dog enzyme and the only one form (corresponding to the α form) was present in other enzymes. Thus, the molecular form of the enzyme does not seem to account for the difference in ouabain sensitivity of cardiac (Na^+ + K^+)-ATPase. Lack of correlation between the molecular form and ouabain sensitivity of (Na^+ + K^+)-ATPase has also been reported in rat and dog kidney enzymes [19].

On the other hand, a significant difference in temperature dependence of the enzyme activity was observed between the ouabain-sensitive and -insensitive animals. Wynn-Williams [20] reported that T_d might be affected by changes in the pH, the concentration of protein, and metal ions. In this study, we used imidazole-buffer, the pH of which depends on the incubation temperature. But this is not a serious problem, because the optimal pH of all the enzymes is the same, and broad (pH 6.6 to 8.0) (data not shown). Furthermore, similar conditions were used for each enzyme assay. Therefore, it is unlikely that these factors contributed to the difference between the T_d values of the ouabain-sensitive and -insensitive enzymes.

Since it is generally accepted that T_d is attributed to lipid–lipid or lipid–protein interaction [4–6, 21, 22], the present finding suggests that there is a difference

Table 1. Temperature at discontinuity (T_d) and activation energies of cardiac (Na^+ + K^+)-ATPases from various species*

Animal species	T_d (°)	Activation energy (kJ/mole)	
		Above T_d	Below T_d
Bovine	30.3 ± 0.3†	32.6 ± 2.7	102.1 ± 3.4
Dog	29.1 ± 1.0‡	50.9 ± 5.1	108.5 ± 1.7
Guinea pig	28.7 ± 0.8‡	40.1 ± 1.7	98.8 ± 3.8
Rabbit	31.2 ± 0.9†	41.5 ± 4.7	87.4 ± 3.6
Rat	25.4 ± 0.7	46.6 ± 4.1	98.4 ± 2.7
Mouse	23.9 ± 1.2	41.0 ± 3.8	105.9 ± 10.8

* The experiment of Arrhenius plots was repeated three to six times using at least two different enzyme preparations for each enzyme; the T_d value and activation energy are shown as means ± S.E.M. of the separate Arrhenius plots.

† $P < 0.01$, compared to rat and mouse (Student's *t*-test).

‡ $P < 0.05$, compared to rat and mouse.

between interactions of cardiac ($Na^+ + K^+$)-ATPase with lipids in the ouabain-sensitive and -insensitive animals. In accord with this suggestion, we found a significant difference in phospholipid composition between the ouabain-sensitive and -insensitive enzymes in a preliminary experiment. However, when the ouabain-sensitive enzymes were classified further into highly sensitive (bovine and dog) and moderately sensitive (rabbit and guinea pig) (Fig. 1), there was no change in T_d among these enzymes (Table 1). Therefore, the lipid environment of cardiac ($Na^+ + K^+$)-ATPase may not be a crucial determinant of species differences in enzyme ouabain sensitivity, though it may be responsible for the insensitivity of rat and mouse enzymes to ouabain inhibition.

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