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Changes in the Fluidity of Myocardial Membranes during Hibernation: Relationship to Myocardial Adenosinetriphosphatase Activity

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SUMMARY

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The activity of both myocardial ouabain-sensitive ($Na^+ + K^+$)-ATPase and ouabain-insensitive Mg^{2+}-ATPase is reduced in the hearts of winter-hibernating ground squirrels compared to the activity in the hearts of awake–active animals sacrificed during the summer. ($Na^+ + K^+$)-ATPase preparations from the nonhibernating animals show non-linear Arrhenius kinetics, with a marked increase in the Arrhenius activation energy (E_a) below 16°C. During hibernation this pattern of temperature dependence probably does not change significantly, but the level of enzyme activity below 16°C is now too low to measure with confidence. Conversely, even the reduced level of myocardial Mg^{2+}-ATPase from the hearts of animals killed during hibernation continues to display the linear Arrhenius kinetics that were observed in these myocardial preparations from active summer animals. Biophysical studies of the whole myocardial membrane preparations by differential scanning calorimetry and examination of the total lipid extract from these membranes by electron spin resonance spectroscopy after labeling of the lipids with 16 NS suggest that the myocardial membrane is in a more fluid state when the animals are in hibernation. However, only a small percentage of the lipids of the membranes is involved in the thermal transitions observed, and the lack of coincidence of the temperature for these transitions and the parameters of activation energy for both myocardial adenosinetriphosphatases indicates that the activities of ($Na^+ + K^+$)-ATPase and Mg^{2+}-ATPase are insulated against this change in lipid structure—possibly by a domain of boundary lipids possessing thermal properties different from those of the bulk phase. During hibernation there is a significant increase in the 18^2 "6 fatty acid (linoleic) content of myocardial membranes.

INTRODUCTION

Recently, we have described the seasonal variation in the positive inotropic response to the naturally occurring cardiac glycoside ouabain and to the semisynthetic glycoside actodigin which occur in both atrial and papillary muscle strips obtained from the hearts of hibernating and nonhibernating ground squirrels (*Spermophilus richardsonii*) (1, 2). During the summer months when the animals are awake and active, their body temperature is near 37°C, their heart rate is high, and isolated myocardial muscle preparations display a marked increase in isometric twitch tension when subjected to concentrations of ouabain between 8×10^{-9} and 1×10^{-7} M. Conversely, during the deep torpor of winter hibernation when the body and organ temperature of the ground squirrel falls to below 5°C, their heart rate is greatly decreased and isolated myocardial muscle preparations from these animals now show a significantly reduced inotropic response to both ouabain and actodigin. This depression in pharmacological response occurs concurrently with a significant reduction in myocardial ($Na^+ + K^+$)-ATPase activity (EC 3.6.1.3) and a decrease in the ouabain-binding capacity of the myocardial membranes (3). The latter results imply that the loss in positive inotropic response during hibernation occurs at a time when the putative pharmacological receptor for cardiac glycosides, i.e., myocardial membrane ($Na^+ + K^+$)-ATPase (4, 5), is also reduced, thus suggesting that the pharmacological changes during hibernation may be a membrane-mediated effect directly involving ($Na^+ + K^+$)-

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ATPase. However, other changes in the function of either the drug–receptor system or the myocardial muscle contractile mechanism cannot be excluded.

Because it is known that membrane-associated ($Na^+ + K^+$)-ATPase is a lipid-modulated enzyme whose function can be altered by changes in the lipid composition and structure of the membrane matrix (6–8), we have explored this aspect of the problem by examining the relationship between myocardial ($Na^+ + K^+$)-ATPase and Mg^{2+}-ATPase activities of membranes from summer-active and winter-hibernating ground squirrels. Our results suggest that in addition to the marked reduction in the specific activities of ($Na^+ + K^+$)-ATPase and Mg^{2+}-ATPase which occur during hibernation (3), a significant change also occurs in the physical properties and lipid composition of the myocardial membranes of the ground squirrel during these widely different pharmacological states.

MATERIALS AND METHODS

The collection from the field and the maintenance of a colony of Richardson ground squirrels during hibernation have been fully described in previous publications (9, 10). The active-summer animals were killed during June and July, and the winter-hibernating animals during the following January and February, at least 75 days after the initiation of the first hibernation cycle in captivity. Hibernation was monitored by the "sawdust" method of Lymann (11), and no animal was sacrificed by decapitation unless it was at least 5 days into a hibernation/arousal cycle. The LiBr extraction procedure used in our method of myocardial membrane preparation and the assay of ($Na^+ + K^+$)-ATPase and Mg^{2+}-ATPase by the coupled spectrophotometric procedure utilizing the oxidation of NADH have also been described in full (3, 12). Cardiac tissues (left and right ventricles) were pooled from at least three animals for each enzyme preparation, and assays were always carried out in duplicate.

It should be recalled that our assay procedure permits the simultaneous determination of ouabain-insensitive "basal" Mg^{2+}-ATPase, "cation-stimulated" ($Na^+ + K^+$)-ATPase, and "ouabain-inhibitable" ($Na^+ + K^+$)-ATPase at any one time or temperature. ($Na^+ + K^+$)-ATPase is defined as the difference between the activity in the presence of 80 mM Na^+ + 20 mM K^+ ± 2 mM ouabain (3, 12). Specific activity was defined as micromoles NADH oxidized (equivalent to micromoles ATP hydrolyzed) per milligram membrane protein per hour at 37°C. The effect of temperature on enzyme velocity was determined by estimation of enzyme reaction rates at various temperatures using a Gilford 2400 spectrophotometer fitted with an externally controlled temperature-jacketed cell holder. Where required, we again used a computer-assisted analysis of the data to determine (by regression analysis) the best single- or two-line plot which fitted a family of points obtained between 10 and 37°C. This analysis yields values for the apparent (Arrhenius) activation energy for the hydrolysis of ATP by myocardial adenosinetriphosphatase; the details of the analysis have been published previously (13) and do not differ significantly from the analytical procedure employed by McMurchie and Raison (14, 17, 30). The values for the Arrhenius activation energies so obtained are given as kilocalories per mole.

Differential scanning calorimetry. Myocardial membrane preparations enriched in ($Na^+ + K^+$)-ATPase were pelleted at 100,000*g* for 60 min using a Beckman L2-65B refrigerated ultracentrifuge. The pellets, which were all about 20 mg wet weight, were sealed in 20-μl-capacity gold pans, and the thermotropic behavior of the membranes was determined using a Perkin Elmer DSC-2 differential scanning calorimeter. The thermal scan rate was 10°C/min at operating sensitivities of either 0.2 or 0.5 mcal/s as indicated in the legends to the figures. The dry weight of the samples was determined after DSC analysis, and all samples were about 5 mg in weight, of which less than 25% was total lipid for both active-summer and winter-hibernating animals.

Extraction and analysis of myocardial membrane lipids. After dilution in 25 vol of Tris buffer (25 mM Tris–HCl at pH 7.4), the myocardial membranes were pelleted by centrifugation, extracted with 2:1 chloroform:methanol using the method described by Bligh and Dyer (16), and stored at −20°C in chloroform under nitrogen.

Quantitative fatty acid analysis was performed on the extracted lipids after esterification in 1% H_2SO_4 in methanol at 70°C for 2 h, by gas–liquid chromatography using a Hewlett Packard gas chromatograph (Model 5840A) equipped with a printer plotter integrator and a flame ionization detector and a hybrid column (Supelco NS1-1833), temperature programmed from 125 to 225°C at 4°C/min. The injection temperature was 200°C and the detector temperature was 300°C; the carrier gas was N_2 at a flow rate of 20 ml/min. All determinations were carried out in triplicate or more, and the results of individual determinations agreed within 2%. The cholesterol content of the total lipid extract was determined by the micromethod of Magee *et al.* (17) which also employs gas–liquid chromatography.

Electron spin resonance spectroscopy. The lipids in chloroform:methanol were dried under N_2 and resuspended by brief sonication in 0.1 M Tris–acetate buffer containing 5 mM EDTA (pH 7.2). The suspension of lipids was then labeled with a nitroxide derivative of methyl stearate [3-oxazolidinyloxy-2-(14-carbmethoxytetradecyl)-2-ethyl-4,4 dimethyl] henceforth referred to as 16 NS, in the proportion of 1 mol of spin label for each 75 mol of phospholipid. Spectra were obtained with a Varian E4 spectrometer fitted with a temperature-controlled cell holder (15), and the empirical motion parameter τ_0 was determined by the method described by Mehlhorn *et al.* (18).

RESULTS

The membrane preparations usually contained about 6 mg protein and 1.5 mg lipid. The ouabain-sensitive ($Na^+ + K^+$)-ATPase activity of these preparations was about 50% of the total activity determined in the presence of Mg^{2+}, Na^+, and K^+ ions. After extraction with LiBr these preparations are substantially free of endoplasmic reticulum and mitochondrial membranes; the ouabain-sensitive ($Na^+ + K^+$)-ATPase is enriched about fourfold

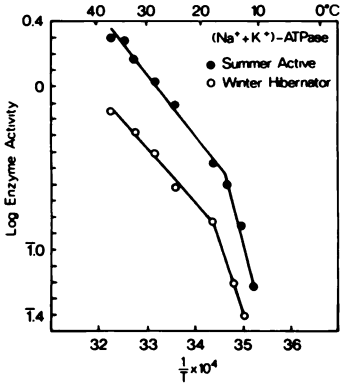

FIG. 1. *Temperature–activity relationship of myocardial (Na^+ + K^+)-ATPase of summer-awake and winter-hibernating ground squirrels*

Data displayed as an Arrhenius plot of log enzyme activity versus the reciprocal of the absolute temperature. The enzyme activity is given as μmol ATP hydrolyzed/mg membrane protein/h at the various experimental temperatures. The number of animals (N) was 8 for the summer-awake animals (●) and 7 for the winter-hibernating (○) animals. The standard errors of the means (±SE) are small and lie within the dimensions of the points, except for the points below 16°C from winter-hibernating animals, where the enzyme activity decreased so sharply that the levels were at the limits of detectability. The values for the Arrhenius activation energies obtained from this data are given in Table 1.

over that before treatment with a chaotropic agent (12, 19).

Enzyme kinetics. The effect of temperature on myocardial (Na^+ + K^+)-ATPase is shown as an Arrhenius plot in Fig. 1, where it can be seen that this ouabain-sensitive enzyme prepared from the myocardium of awake-summer animals shows a marked temperature dependence with a clearly nonlinear relationship between rate and temperature. There is a very sharp decrease in enzyme activity at about 16°C, below which temperature the level of activity is greatly reduced. While it can be seen from Table 1 and Fig. 1 that the activity of (Na^+ + K^+)-ATPase was very much less in the preparations from the myocardia of animals killed during winter hibernation, thus confirming our earlier results (3), the pattern of temperature dependence probably did not change significantly during this physiological state, although the decline in activity at 16°C was so sharp that below this temperature we were unable to determine the activity of myocardial (Na^+ + K^+)-ATPase with much accuracy. Above 16°C the Arrhenius activation energy (E_a) for myocardial (Na^+ + K^+)-ATPase from winter-hibernating animals was identical to that obtained from awake-summer animals (Table 1).

Conversely, a very dissimilar pattern of temperature response was found with ouabain-insensitive myocardial Mg^{2+}-ATPase (Fig. 2). Whether this enzyme was assayed in myocardial membranes prepared from either active-summer animals or ground squirrels killed after more than 75 days in hibernation, all preparations showed linear Arrhenius plots which yielded single values for E_a over the entire temperature range examined (10–37°C). The values obtained for the E_a of active-summer and winter-hibernating ground squirrel myocardial Mg^{2+}-ATPase were identical in each biological state and are given in Table 1. These values were always considerably less than the values obtained from myocardial (Na^+ + K^+)-ATPase irrespective of the animals' biological state or the temperature range of the enzyme assay.

From these experiments, it is clear that the nonlinear Arrhenius profile of myocardial (Na^+ + K^+)-ATPase from summer-active ground squirrels is similar to that of this enzyme prepared from either the brain or the renal cortex of this species (11, 12). Furthermore, it seems unlikely that there are significant changes in the temperature–activity relationships of either ouabain-sensitive or ouabain-insensitive myocardial adenosinetriphosphatases which are due to different physiological states of the animal associated with seasonal variations, although the marked fall in the specific activity of (Na^+ + K^+)-ATPase which occurs during hibernation in the ground squirrel prevents accurate examination of this important property. The temperature–activity relationship of (Na^+ + K^+)-ATPase of this hibernating species therefore may not differ significantly from that of the (Na^+ + K^+)-ATPase obtained from a variety of tissues from nonhibernating species (20).

However, the absence of seasonal variation in the activation energies of myocardial membrane-associated adenosinetriphosphatases does not exclude the possibility that some changes occurred in the physical properties of these membranes during hibernation (20, 21). We therefore examined the physical properties of these myocardial membranes for any seasonal variation by several biophysical procedures.

TABLE 1

Seasonal variation in the specific activity and activation energies of myocardial adenosinetriphosphatases in cardiac membranes[a] from Spermophilus richardsonii

Enzyme	Summer-awake animals			Winter-hibernating animals		
	Sp act	High range[b]	Low range[b]	Sp act	High range[b]	Low range[b]
(Na^+ + K^+)-ATPase	2.2 ± 0.1	16.7 ± 1.0	49.1 ± 2.8	0.71 ± 0.1	17.6 ± 1.2	32.1 ± 9.7
Mg^{2+}-ATPase[c]	3.9 ± 0.3	12.4 ± 0.4		0.94 ± 0.1	12.7 ± 0.4	

[a] Cardiac membranes were prepared by the LiBr extraction procedure described in Materials and Methods and Refs. 3 and 19. Activation energies (E_a) given as kcal/mol. Specific activities given as μmol ATP hydrolyzed/mg membrane protein/h at 37°C. All data are the means of at least seven experiments.

[b] High-range activation energy (E_{a_I}) obtained between 16 and 37°C; low-range activation energy ($E_{a_{II}}$) obtained below 16°C.

[c] The single value for activation energy of Mg^{2+}-ATPase indicates that the data could best fit a single straight line as determined by regression analysis (see Ref. 13).

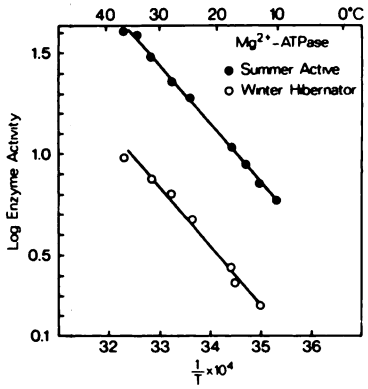

FIG. 2. *Temperature-activity relationship of myocardial Mg^{2+}-ATPase of summer-awake and winter-hibernating ground squirrels*

Data displayed as an Arrhenius plot of log enzyme activity versus the reciprocal of the absolute temperature. The units of enzyme activity are as in Fig. 1. The number of animals (N) was 7 for the summer-awake animals (●) and 7 for the winter-hibernating animals (○). The standard errors of the means (±SE) are small and lie within the dimensions of the points. The Arrhenius plots from both groups of animals are linear throughout the temperature range examined and yield identical values for their activation energy; the values for E_a are given in Table 1.

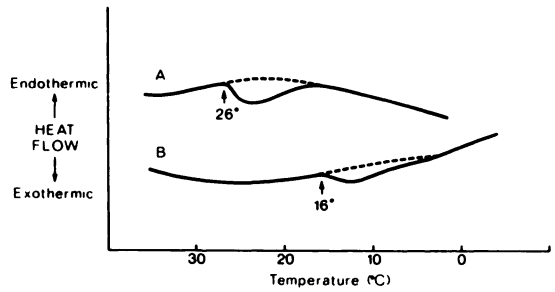

FIG. 3. *Thermal transitions of adenosinetriphosphatase-containing membranes prepared from the hearts of summer-awake (A) and winter-hibernating (B) ground squirrels*

The differential scanning calorimetry traces (DSC) were obtained at a cooling rate of 10°C/min. The operating sensitivities were recorded at 0.2 mcal/s for preparations from summer-awake animals and 0.5 mcal/s for preparations from winter-hibernating animals. Exotherms which commenced at 26°C were observed in the cardiac membranes from animals from group A and at 16°C in cardiac membranes of animals from group B.

Differential scanning calorimetry. The upper scan (A) in Fig. 3 shows that the myocardial membranes obtained from awake-summer ground squirrels (which had a body and organ temperature near 37°C) undergo an exothermic transition which commences at 26°C, has a midpoint at about 21°C, and is complete by 16°C. By contrast, no exotherm commenced at this temperature in the myocardial membranes of the ground squirrel sacrificed during the deep torpor of hibernation. However, a distinct exothermic transition now commenced at 16°C and extended for at least 8°C lower.

The latter result is shown in the lower scan (B) in Fig. 3; the temperature of onset of this particular exotherm corresponds exactly with the temperature at which there is a sharp decline in the activity of myocardial (Na^+ + K^+)-ATPase from both summer-active and winter-hibernating ground squirrels.

Because these exothermic transitions are still evident after thermal denaturation of membrane proteins (observable by a large irreversible endotherm at 57°C), we attribute these transitions to reversible changes in the membrane lipids rather than to any protein components of the membrane. The enthalpy of the transition can be calculated from the heat involved in the exotherm and the amount of lipid in the membrane sample. From the enthalpy of the transition of a pure phospholipid (22), we calculate that no more than 2% of the membrane lipid is involved in the transition observed between 37 and 0°C in myocardial membranes from summer-active animals. With the membranes from hibernating animals, the amount of lipid may be considerably less than 2%.

Electron spin resonance spectroscopy. To further explore the possible involvement of membrane lipids in the function of myocardial (Na^+ + K^+)-ATPase in hibernation, thermally induced changes in lipid properties of myocardial membranes of awake-active and hibernating ground squirrels were examined by a spin labeling procedure similar to that previously employed by McMurchie and Raison to study the role of membrane lipids during hibernation in the Echidna (*Tachyglossus aculeatus*) (14). Preliminary experiments demonstrated that when the spin label 16 NS was infused into our cardiac membrane preparations, the motion of this label was markedly anisotropic and the label was significantly immobilized, indicating that 16 NS was associated more with membrane proteins than with the lipids. By contrast, when 16 NS was infused into aqueous dispersions of the extracted lipids from these membranes, the spin label exhibited rapid and almost isotropic motion over the temperature range examined. Therefore electron spin resonance spectroscopy was routinely carried out on the extracted lipids rather than the whole cardiac muscle membrane preparations. The thermally induced changes in the empirical motion parameter τ_0 were recorded, as these changes in the temperature coefficient of spin label motion have been correlated with changes in the molecular ordering of the host lipids (18, 23). The changes in this property of the spin label 16 NS at 29°C for myocardial membranes from summer-active ground squirrels and at 18°C in myocardial membranes from winter-hibernating ground squirrels shown in Fig. 4 are therefore considered indicative of temperature-induced changes in molecular ordering of the lipids of myocardial membranes. The temperatures at which these changes occur are very close to those at which exothermic transitions were found to occur in whole membrane preparations from the myocardia of these animals and reinforce our view that both these phenomena are reflections of lipid-dependent processes.

Seasonal variation in the total fatty acid composition of myocardial membranes. In addition to seasonal changes in the physical properties of the myocardial membranes of the ground squirrel, the data given in Table 2 demonstrate that there are major seasonal changes in the lipid composition of these membranes. Here it can be seen that as the percentage of total saturated fatty acids of the myocardial membranes has decreased during the hibernation state, there is a signif-

icant increase in the percentage of the total unsaturated fatty acids. This is accounted for by an almost twofold increase in the ω6-polyunsaturated fatty acids, which increased from 15 to 26% of the total fatty acid content of the membrane.

In particular, the amount of linoleic acid (i.e., 18^2 ω6) in these myocardial membrane preparations increased from about 6% of the total fatty acids in awake-summer control animals to about 26% of the total fatty acids in the myocardial membranes from animals killed after more than 75 days of hibernation (Table 3). On the other hand, the cholesterol content of the myocardial membranes of winter-hibernating ground squirrels remained similar to that found in these membranes from awake active-summer animals.

However, because of the elevated levels of unsaturated fatty acids that were found, it is reasonable to believe

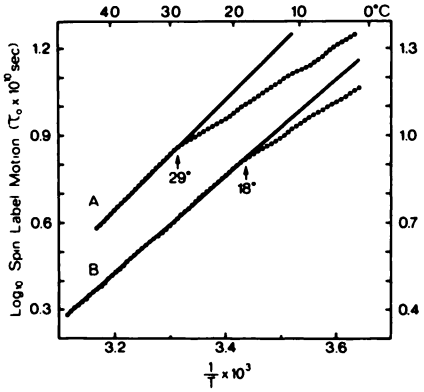

FIG. 4. *The effect of temperature on the motion of the spin label 16 NS intercalated into a dispersion of lipids extracted from adenosine-triphosphatase-enriched membranes prepared from the hearts of summer-awake (A) and winter-hibernating (B) ground squirrels*

Deviation from the linear relationship between the logarithm of the motion parameter of the label (log $\tau_0 \times 10^{10}$ s) and the reciprocal of the absolute temperature is indicative of a change in order of the host lipids. The temperature at which deviation occurred was estimated by assuming the data fit two straight lines and determining the intersection of the lines which gave a minimum for the residual sum of the squares for both lines by the method of Pollard (30). Deviation from linearity occurred at 29°C in the lipids extracted from the myocardial membranes of animals in group A and at 18°C in the lipids extracted from the myocardial membranes of animals in group B.

TABLE 2

Seasonal variation in the fatty acid and cholesterol composition of myocardial membranes[a] from summer-awake and winter-hibernating ground squirrels

	% Composition total lipids	
	Summer-awake	Winter-hibernating
Fatty acids		
Total saturated	39.8	32.3
Total unsaturated	59.2	67.2
Total monounsaturated	37.5	38.6
Total ω6 polyunsaturated	15.4	25.8
Total ω3 polyunsaturated	6.3	2.8
Total unknowns	0.9	0.7
Cholesterol	5.3	4.6
Ratio cholesterol:total ω6 polyunsaturated fatty acids	2.9	5.6

[a] Results obtained from membrane preparations derived from the pooled tissues of at least three animals. The values are the means of three determinations which agreed within 2%.

TABLE 3

Effect of the LiBr extraction procedure on the percentage composition of the major fatty acids of myocardial membranes[a]

Fatty acid	Summer-awake animals		Winter-hibernating animals	
	Before treatment	After treatment	Before treatment	After treatment
14^0	2.0	1.1	0.4	0.3
16^0	23.6	22.1	15.0	13.9
18^0	18.0	16.2	20.4	19.5
16^1	2.0	2.5	1.1	1.0
18^1	17.5	21.6	20.1	20.0
18^2	5.7	6.0	24.8	28.7
20^4	6.2	5.9	9.3	8.9
22^1	8.7	10.9	2.2	1.2
22^5	4.1	3.4	0.4	0.5
22^6	3.1	2.1	1.3	1.7
Total	90.9	91.8	95.0	95.7

[a] Summer control animals sacrificed during July 1978; winter-hibernating animals sacrificed during February 1979. The membranes were prepared from the pooled tissues of at least five animals and examined before and after treatment with LiBr as described by the method of Charnock *et al.* (3). The values are the means of three determinations which agreed within 2%.

that an overall increase in the fluidity of the myocardial membranes of the ground squirrel *Spermophilus richardsonii* had occurred during hibernation.

Comparison of the temperature coefficients of spin label motion (τ_0) at 37°C, given in Fig. 4, shows that a decrease occurs in the myocardial membranes from winter-hibernating animals which is consistent with an increase in fluidity. This increase in fluidity is equivalent to the change observed for a temperature shift of 10°C.

These observations are in agreement with our finding of thermal transitions at lower temperatures by both differential scanning calorimetry and electron spin resonance in myocardial membranes from winter-hibernating animals.

That the seasonal changes in the percentage composition of the total membrane fatty acids are independent of the treatment of the cardiac membranes by the lithium bromide which is used to enrich the preparations with ($Na^+ + K^+$)-ATPase is also shown by the data given in Table 3. Here we present an analysis of the major fatty acid components of myocardial membrane preparations from awake-summer and winter-hibernating animals, both before and after extraction with lithium bromide. It is evident that this chaotropic agent which is used to solubilize muscle proteins has no major effect upon the fatty acid composition of the myocardial membranes. The relatively small variations in the percentage composition of any individual fatty acid represent the normal experimental variation between samples and could not account for the significant change in fatty acid composition between winter-hibernating and summer-awake animals reported above.

DISCUSSION

The role of ($Na^+ + K^+$)-ATPase in the inotropic action of the cardiac glycosides continues to be the subject of much research and discussion (4, 5, 24, 25). However, the

large seasonal variations in the positive inotropic response to the cardiac glycosides ouabain and actodigin that we have observed in myocardial muscles from nonhibernating and hibernating ground squirrels (*Spermophilus richardsonii*) provide a further opportunity to explore the properties of this lipid-dependent, membrane-associated enzyme system in relation to the pharmacological action of these clinically important drugs (1–3).

While previous studies by Akera *et al.* (26, 27) and Charnock *et al.* (12, 28) have implicated membrane lipids in the association of cardiac glycosides with $(Na^+ + K^+)$-ATPase preparations from brain, more recent studies by Choi and Akera (29) have shown that although the *association* rates for ouabain binding to cardiac preparations are not greatly altered by treatments designed to alter membrane lipids, increased purification of cardiac $(Na^+ + K^+)$-ATPase by treatment with deoxycholate, sodium iodide, and glycerol leads to increased *dissociation* of bound [^{3}H]ouabain from the drug–receptor complex, particularly in the presence of K^+. These results might be explained by the presence of a "lipidic barrier" which regulates the interaction of inotropic agents with $(Na^+ + K^+)$-ATPase (27, 28).

We have explored this aspect of the problem by experiments designed to examine the relationship between myocardial $(Na^+ + K^+)$-ATPase activity, the lipid composition of myocardial membranes, and some physical properties of these membranes prepared from both pharmacologically responsive awake-active ground squirrels killed during the summer and pharmacologically nonresponsive winter-hibernating animals (1, 2). Our experiments show that the temperature–activity relationship of myocardial $(Na^+ + K^+)$-ATPase of the awake-active ground squirrel (*Spermophilus richardsonii*) is similar to that of both brain and kidney $(Na^+ + K^+)$-ATPase preparations from these animals (9, 10). Although the temperature–activity relationship of $(Na^+ + K^+)$-ATPase can be influenced by membrane lipids in some systems (6–8), this parameter probably does not undergo any significant change when the ground squirrel enters into hibernation. Therefore, in this regard the ground squirrel (*Spermophilus richardsonii*) cannot be distinguished from such nonhibernating species as the rat, the rabbit, or the sheep (20).

It should be emphasized that the absence of seasonal change in the activation energy of myocardial $(Na^+ + K^+)$-ATPase does not mean that other changes in the function of this enzyme cannot play an important role in the seasonal variation in the pharmacological effect of this drug–receptor system (1, 2).

However, it does suggest that the activation energy of $(Na^+ + K^+)$-ATPase is not directly involved in the marked decrease in the inotropic response to cardiac glycosides that is seen during hibernation (1, 2). From both this and our previous study of seasonal variation in the hydrolytic activity and [^{3}H]ouabain binding of myocardial $(Na^+ + K^+)$-ATPase (3), it seems much more likely that the decrease in inotropic effect of the cardiac glycosides during hibernation is associated with the marked decrease in enzyme activity which occurs during this biological state. This of course, stresses the direct relationship between the number of drug–receptor binding sites available and the magnitude of the pharmacological response obtained.

The temperature–activity relationship of myocardial Mg^{2+}-ATPase is more difficult to access, for although the Arrhenius plots of this relationship are linear and without any seasonal variation, the precise biological role of this membrane associated enzyme has yet to be defined. However, the constancy of this result with myocardial Mg^{2+}-ATPase is very similar to our earlier findings with membrane preparations of this enzyme from the brain and renal cortex of *Spermophilus richardsonii* (9, 10). Nevertheless, it is apparent from the data given in Table 1 that the activation energy for myocardial Mg^{2+}-ATPase is less than any value obtained for myocardial $(Na^+ + K^+)$-ATPase irrespective of either the season of the year or the temperature range of the enzyme assay.

On the other hand, there are major seasonal changes in the temperature at which thermal transitions occur in these myocardial membranes. The temperature of the transition is not an intrinsic property of the fatty acid chains of those membrane lipids which constitute a domain of solidification, but a product of the interaction of the lipids in the domain with those of the bulk lipid phase. Thus, the decrease in the temperature of the transition is explicable in terms of the increase in unsaturation of the bulk membrane lipids even if the composition of the melting domain remains unchanged. Coincident with the 10°C lowering of the transition temperature, there was an increase in membrane fluidity, determined as the decrease in the motion parameter (τ_0) of the spin label by an amount equivalent to a 10°C shift in temperature. Thus, the fluidity of the membrane lipids at the transition remains constant, which suggests that the temperature of the transition is a result of the interaction between the melting properties of the lipids within the domain undergoing the transition and the fluidity of the host lipid matrix.

While a lowering of the transition temperature occurs in the myocardial membrane from winter-hibernating ground squirrels and there is an increase in the fluidity of these membranes, this change does not influence the temperature response of either myocardial adenosinetriphosphatase examined. This is understandable considering the very small amount of the total membrane lipid involved in the transition. Hence, the functions of myocardial $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase are apparently insulated from the effects of increased fluidity of the bulk lipids of the myocardial membrane.

Thus, while there is a significant reduction in the amount of myocardial $(Na^+ + K^+)$-ATPase, and an equally significant fall in the number of [^{3}H]ouabain binding sites in the myocardial membrane preparations during hibernation (3), the seasonal changes in the pharmacological response to cardiac glycosides that we reported before (1, 2) may also be associated with a general increase in the "fluidity" of the myocardial membranes rather than in any specific alteration in the biochemical properties of the drug–receptor $(Na^+ + K^+)$-ATPase itself. The general concept that the lipid membrane matrix surrounding a drug–receptor protein, and particularly that a lipid "barrier" around the $(Na^+ + K^+)$-ATP-

ase system (27, 28), plays an important role in the dose–response relationship of the cardiac glycosides deserves further investigation.

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