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What role for membranes in determining the higher sodium pump molecular activity of mammals compared to ectotherms?

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Abstract The major body organs of mammals have sodium pumps that turn over energy (ATP) three to four times faster than those of ectotherms, at the same temperature. To examine if membranes play a role in these differences in molecular activity, membrane cross-over experiments were performed using two representative species, *Rattus norvegicus* and *Bufo marinus*. Microsomal membrane of kidney and brain displayed characteristic molecular activity differences (three- to four-fold) between the species. These molecular activity differences could be removed by delipidation. Pre-existing molecular activities and differences could be restored when reconstituted with original membrane. Using the same reconstitution method, species membrane cross-over experiments resulted in toad sodium pumps in rat membrane significantly increasing (≈30–40%) and rat sodium pumps in toad membrane significantly decreasing (≈40%) activities in both kidney and brain. Analysis of membrane composition showed reduced cholesterol content and differences in the fatty acids of phospholipids with higher overall unsaturation in the mammal. The scope for membranes to determine protein performance and its broader implications for metabolism are discussed.

Key words Endotherms · Na^+K^+-ATPase · Polyunsaturation · Reconstitution · Metabolism

Abbreviation *DOC* deoxycholate

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Introduction

The development of endothermy (increased body heat production) in mammals conveyed the major advantage of metabolic flexibility to respond to environmental demands but at the cost of increased energy requirement (Bennett and Ruben 1979; Ruben 1996). The evolution of endothermy increased the cost of homeostasis by increasing processes such as the leakage of H^+ across inner mitochondrial membranes, and Na^+ and K^+ across cell membranes that together can account for a large proportion (up to 50%) of the resting energy requirements of mammals (Brand et al. 1991; Hulbert and Else 1990). The extra and significant cost to homeostasis of moving Na^+ and K^+ across cell membranes is reflected in the maximal enzymatic activity of the ubiquitous sodium pump (i.e. Na^+K^+-ATPase).

The major organs and tissues of mammals possess greater Na^+K^+ATPase activity than those of reptiles, amphibians and fish (i.e. ectothermic vertebrates) at the same temperature (Else et al. 1996). However, this extra work appears not to be associated with an increase in sodium pump number. Analysis of sodium pump concentration of major organs has shown consistent values that are dependent upon the organ examined (e.g. in pmoles per g tissue; 250 pmoles $\cdot g^{-1}$ for skeletal muscle, 500 pmoles $\cdot g^{-1}$ for liver, 900 pmoles $\cdot g^{-1}$ for heart and 8,000 pmoles $\cdot g^{-1}$ for brain and kidney) rather than the metabolism of the animal (Else et al. 1996).

Hence, the extra work performed by mammalian sodium pumps appears to be derived from an increase in power output or molecular activity (derived by dividing maximal enzyme activity, Na^+K^+ATPase, by sodium pump density) rather than an increase in the number of sodium pumps for a given organ. The molecular activity of sodium pumps is approximately 8,000 ATP $\cdot min^{-1}$ in mammals and 2,500 ATP $\cdot min^{-1}$ in ectotherms at 37 °C (Else et al. 1996). Therefore, to maintain Na^+/K^+ homeostasis, mammals have matched the increased ion permeability of their membranes

with an increase in the power, rather than number, of their sodium pumps.

The aim of this study was to begin to investigate the molecular activity increase in the sodium pumps of mammals compared to those of ectotherms. Specifically, we wanted to examine any potential role played by membranes. To do this, sodium pumps in two major organs i.e. kidney and brain in a representative mammal, *Rattus norvegicus*, and ectotherm, *Bufo marinus*, were examined. The study involved (i) determining if the molecular activity differences persisted in membrane preparations (versus those previously found in tissue biopsies/homogenates), and if so, (ii) determining the effect of membrane cross-over experiments where the sodium pumps of each animal are subjected to the membrane environment of the other species.

Materials and methods

Animals

The species used in this study included the rat (*R. norvegicus*, male, Sprague-Dawley strain, aged 12–14 weeks, body weight range 200–500 g) and cane toad (*B. marinus*, 100–250 g). Both species were housed under 12:12 h light:dark conditions, with free access to food and water at 25–30 °C for toads and 20–25 °C for rats. Species choice was based on similar body masses, the thermal tolerance of *B. marinus* (it can been maintained at 37 °C for several weeks with no adverse effects) and previous work showing differences in molecular activity in organs of the two species (Else et al. 1996). All animal experiments were approved by the University of Wollongong animal ethics committee.

Materials

^{3}H-ouabain (0.54 TBq · mmol^{-1}, 98.3% purity) in ethanol and ^{125}I (0.629 TBq · mg^{-1}) were obtained from Dupont NEN. ^{3}H-ouabain was removed from ethanol under a stream of nitrogen gas and resuspended in pure water at 7.4 MBq · ml^{-1}. The scintillation cocktail (Hionic-Fluor) was from Packard. ATP (special quality) was from Boehringer Mannheim. Ouabain was purchased from ICN. Sodium deoxycholate ($C_{24}H_{39}O_4Na$, 98.5%) was from BDH and Iodogen was from Sigma Chemicals. All standard reagents used were of analytical grade.

Sodium pump densities and Na^+K^+ATPases

Kidney and brain were chosen for this study because of their major contributions to resting metabolism and high sodium pump concentrations. All Na^+K^+ATPase activities were maximal elicitable activities measured at 37 °C, performed as previously described (Else et al. 1996). Microsomes were prepared according to the procedure described by Liang and Winter (1976). Sodium pump density was determined by incubating 10–20 µl of 1 mg · ml^{-1} microsomal preparation for 40 min at 37 °C in 200 µl of 5 mmol · l^{-1} $MgCl_2$, 10 mmol · l^{-1} KH_2PO_4, pH 7.4 plus 10^{-6} mol · l^{-1} ouabain including 7.4×10^3Bq ^{3}H-ouabain in Millipore Ultrafree-MC 30,000 NMWL Eppendorf filters. Parallel ouabain incubations at 10^{-2}mol · l^{-1} were used to determine nonspecific binding. Membranes with radiolabelled sodium pumps were centrifuged (at 2,700 *g* for kidney and at 5,200 *g* for brain, Eppendorf 5417R) at 4 °C for 40 min including 3 × 50 µl washes of cold buffer (no ouabain). ^{3}H-activity suspended on filters (after being removed from their plastic housing) was counted on a Wallac 1409 LSC with DPM correction. Sodium pump density was calculated as nmol · g^{-1} of protein. Protein was assayed by the Lowry method (Lowry et al. 1951), with bovine serum albumin as a standard.

Detergent treatments

Detergent treatments involved adding 0.5 ml (1 mg protein · ml^{-1}) microsomal membrane to 0.5 ml of detergent buffer consisting of Tris (0.25 mmol · l^{-1})-EDTA (2 mmol · l^{-1})-ATP (3 mmol · l^{-1}), with varying concentrations of sodium deoxycholate (DOC) detergent (i.e. 12 concentrations between 0 and 10 mg · ml^{-1}), pH 7.6 for 15 min at room temperature (22 ± 2 °C). Detergent treatment was followed by Na^+K^+ATPase activity measurement.

Membrane reconstitution experiments

Membrane reconstitution occurred after initial detergent treatment of microsomes to reduce molecular activities of both rat and toad to similar low levels. Microsomes were divided into two fractions, Fraction I consisted of 0.5 ml of 1 mg protein · ml^{-1} microsome plus 0.5 ml of detergent buffer. Detergent concentrations used (see details below) were optimised to maximise the detergent effect without destroying the latent activity of the protein. For each preparation individual delipidation profiles were determined. The combined results of theses delipidation experiments are shown in Fig. 1. At high detergent concentrations and with delipidation carried out in a step wise manner we found clear levels at which relipidations between concentration steps either fully reactivated, to similar levels, or tended to reactivate poorly if at all. The final DOC:protein ratios (mg:mg) used for brain were 4.0 for rat and 4.5

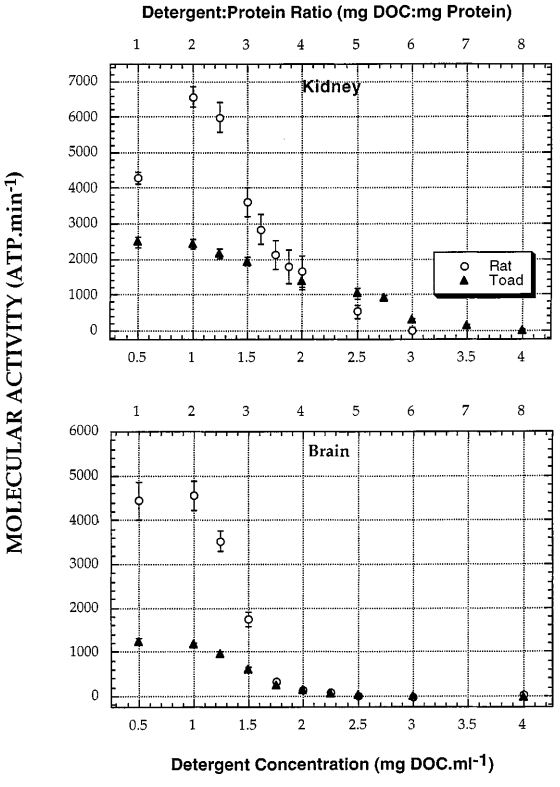

Fig. 1 The effect of increasing deoxycholate (*DOC*) concentration on molecular activity of *R. norvegicus* and *B. marinus* sodium pumps measured in kidney and brain microsomes. Rat organs showed characteristic high mammalian molecular activities compared to the same toad organs. All preparations decreased activity with increasing detergent concentration but the rate of decrease in both rat organs was more rapid resulting in a convergence of rat and toad sodium pump molecular activities at higher detergent concentrations. $n = 9$ kidney and $n = 4$ brain preparations (each preparation used between 10–20 kidneys or brains for each microsomal preparation). Error bars are SEMs

for toad, and for kidney they ranged from 3.5 to 5 for rat and from 3.5 to 7 for toad. Detergent treatment reduced molecular activities down to 8–15% and 9–11% of maximal activities in brain and kidney, respectively. Fraction II consisted of the same microsomal membrane at a concentration of 2 mg protein $\cdot$ ml^{-1} that had been immersed in boiling water for 5 min. Heat treatment destroyed Na^+K^+ATPase activity (verified using Na^+K^+ATPase assay) but did not change the phospholipid fatty acid composition of either organ of rat or toad ($P > 0.05$). Fatty acid analysis of microsomal phospholipids was conducted as described by Pan et al. (1996) using gas chromatography. Cholesterol content of microsomal membranes was measured using Sigma diagnostic kit 352-20 and cholesterol calibration standard.

Reconstitution involved incubating the 1-ml detergent-treated Fraction I (0.5 mg of total protein) with 2 ml heat treated Fraction II at room temperature (22 $\pm$ 2 °C) for 5 min. This simultaneously decreased detergent concentration to optimal levels, approximately 0.70 mg and 0.75 mg DOC $\cdot$ ml^{-1}, rat and toad respectively, as shown in Fig. 1, in an environment of excess membrane lipid. This membrane addition was optimised in preliminary experiments used to design the reconstitution protocol. These experiments showed a relationship in both species between the amount of Fraction II added and activity recovered; 2 ml Fraction II produced maximal recovery of enzyme activity, with further additions producing no further change in activity (in both species). Microsomal additions of less than 2 ml showed reduced activities.

Reconstituted microsomal membranes were centrifuged at 150,000 *g* at 2 °C for 90 min. In the standard experiment, control tubes (without added Fraction II) were used to determine protein recovery. This procedure was verified using iodinated Fraction I microsomal membranes (prepared by the Iodogen method) and comparing pellet protein yield in the presence and absence of Fraction II. Pellets were resuspended in 700 µl of ATP-Tris-EDTA buffer [25 mmol $\cdot$ l^{-1} Tris (pH 7.6 at 25 °C), 2 mmol $\cdot$ l^{-1} EDTA, 3 mmol $\cdot$ l^{-1} ATP] and Na^+K^+ATPase activity and sodium pump concentration determined.

Results

Na^+K^+ATPase, ^{3}H-ouabain binding and molecular activities measured for kidney and brain microsomes are shown in Table 1. The molecular activities for *R. norvegicus* microsomal membranes are higher than those in *B. marinus* and similar to those previously reported for intact tissue (Else et al. 1996, see Table 1). Molecular activities for rat preparations ranged from 5285 ATP $\cdot$ min^{-1} to 9138 ATP $\cdot$ min^{-1}. Molecular activities for toad preparations ranged from 1548 ATP $\cdot$ min^{-1} to 2922 ATP $\cdot$ min^{-1} (brain and kidney respectively), a three- to four-fold difference. Therefore molecular activity differences between the two species, in both tissues, are conserved in the microsomal membrane preparations.

In order to examine the relationship between the membrane and molecular activity of sodium pumps, a mild detergent treatment was used to disperse the lipid component of the membrane (see Fig. 1). Graded application of detergent to microsomes of rat and toad resulted in similar patterns of decreasing molecular activity at higher detergent concentrations (Fig. 1). However, the decrease in activity of rat microsomes was more rapid, resulting in a convergence of molecular activities at low values (500–1000 ATP $\cdot$ min^{-1}) between the two species.

To test the hypothesis that membrane type (i.e. endo- or ectothermic) contributes to the differences in molecular activity, membrane cross-over experiments were performed. These experiments involved detergent treatment of microsomal membranes followed by reconstitution. In the reconstitution experiments, the detergent-treated sodium pumps (see Materials and methods) were reconstituted with both original and foreign (other species) heat-treated membrane. The heat-treated membrane possessed no Na^+K^+ATPase and retained its fatty acid composition (results not shown). Results of these experiments are shown in Fig. 2.

When delipidated (detergent-treated) membrane was reconstituted with heat-treated membrane of the same

Table 1 Molecular activity of sodium pumps in preparations from kidney and brain of rat and toad (*n* refers to the number of preparations with between 10 and 20 kidneys and brains required for each preparation)

Preparation	Rat			Toad		
	Sodium pump number (nmol $\cdot$ g^{-1} [a]tissue or [b]protein)	Na^+K^+-ATPase activity (µmol Pi $\cdot$ mg^{-1} [a]tissue or [b]protein $\cdot$ h^{-1})	Molecular activity (ATP $\cdot$ min^{-1})	Sodium pump number (nmol $\cdot$ g^{-1} [a]tissue or [b]protein)	Na^+K^+-ATPase activity (µmol Pi $\cdot$ mg^{-1} [a]tissue or [b]protein $\cdot$ h^{-1})	Molecular activity (ATP $\cdot$ min^{-1})
Kidney						
[c]Tissue (biopsy/homogenate)	7.06 $\pm$ 0.73[a] (n = 6)	2.69 $\pm$ 0.14[a] (n = 6)	[d]6354[e]	10.4 $\pm$ 1.7[a] (n = 6)	1.46 $\pm$ 0.22[a] (n = 6)	2340[e]
Microsomal membranes	171 $\pm$ 71[b] (n = 3)	67.3 $\pm$ 2.9[b] (n = 9)	[f]8348 $\pm$ 3200 (n = 3)	432 $\pm$ 54[b] (n = 3)	73.6 $\pm$ 5.0[b] (n = 9)	[f]2922 $\pm$ 345 (n = 3)
Brain						
[c]Tissue (biopsy/homogenate)	5.64[a,g] (n = 9)	3.09 $\pm$ 0.10[a] (n = 5)	9138[e]	10.8 $\pm$ 1.9[a] (n = 6)	0.98 $\pm$ 0.22[a] (n = 6)	2345[e]
Microsomal membranes	330 $\pm$ 64[b] (n = 3)	90.2 $\pm$ 6.4[b] (n = 5)	[f]5285 $\pm$ 928 (n = 3)	846 $\pm$ 163[b] (n = 3)	73.0 $\pm$ 3.5[b] (n = 5)	[f]1584 $\pm$ 370 (n = 3)

[a] or [b] indicates relative to tissue or protein mass respectively
[c] tissue values from (Else et al. 1996)
[d] Molecular activity values were derived by dividing maximal Na^+K^+ATPase activities (nmol Pi $\cdot$ g^{-1} protein or tissue $\cdot$ min^{-1}) by the sodium pump density (in nmol $\cdot$ g^{-1} protein or tissue)
[e] SEM not given as sodium pump number and activity were determined from different preparations
[f] Indicates values where both number and activity were determined on the same preparation
[g] SEM absent due to multiple binding sites

origin, the molecular activity of the delipidated sodium pumps could be re-established to pre-existing levels (83–104% in kidney and brain of both species). Alternatively, reconstitutions carried out using foreign heat-treated microsomal membrane showed a differential effect. The addition of toad membrane to delipidated rat sodium pumps decreased pump molecular activity by 39% in both tissues ($P < 0.031$ and $P < 0.030$, kidney and brain respectively), but addition of rat membrane to delipidated toad sodium pumps increased molecular activity by 42% in kidney ($P < 0.012$) and 31% in brain ($P < 0.007$). These significant changes were unequivocal and occurred in every experiment conducted, in both kidney and brain. Therefore, sodium pumps of either rat or toad performed best under the influence of the rat membrane environment.

The phospholipid fatty acid composition and cholesterol content of the microsomal membranes are shown in Table 2. This analysis shows characteristic differences between kidney and brain, common to both species, as well as species differences. For example, the brain shows characteristic high 22:6n-3 levels compared to kidney, but rat shows significantly higher levels of this and other n-3 fatty acids in both kidney and brain, resulting in significant differences in n-6:n-3 ratios between the rat and the toad organs. In contrast, the toad organs possess more n-9 fatty acids. Because of these and other differences, the rat organs possess higher total levels of unsaturation, with approximately two double bonds per fatty acid versus 1.75 for toad. The fatty acids of the rat organs are also generally deeper in the membrane, with more n-3 and less peripheral n-9 double bonds than, the toad organs. Cholesterol content was found to differ between the two organs, being lower in those of the mammal.

Discussion

The role that membranes may play in determining the three- to four-fold different sodium pump molecular activity (ATP turnover) between endotherms and ectotherms was investigated in two organs (kidney and brain) of two representative species (*R. norvegicus* and *B. marinus*). Using a reconstitution protocol, it was possible to reconstitute the molecular activity of all preparations to 83–104% of original activity after detergent treatment that had reduced molecular activity down to 500–1000 $ATP \cdot min^{-1}$ or between 8–15% of original activity. Reconstitution of *B. marinus* sodium pumps in *R. norvegicus* heat-treated membrane increased molecular activity by up to 40%. In contrast, *R. norvegicus* sodium pumps reconstituted in *B. marinus* heat-treated membrane decreased their activity by a similar percentage. These results support the hypothesis that the membrane environment can influence the molecular activity of the sodium pump and contribute to the difference between rat and toad.

One explanation for these results is that simple dilution of the detergent-treated microsomes and the sub-

Table 2 Relative percentage of phospholipid fatty acid composition and cholesterol content of rat and toad kidney and brain microsomal membranes. Fatty acids less than 1.5% of total fatty acids in both species were not included in the table

Fatty acid	Kidney		Brain	
	Rat ($n = 6$)	Toad ($n = 6$)	Rat ($n = 6$)	Toad ($n = 5$)
Saturated				
C16:0	16.8 ± 0.6	17.1 ± 0.1	20.8 ± 0.9	23.7 ± 0.9[a]
C18:0	16.8 ± 0.7	8.92 ± 0.17[b]	18.1 ± 0.5	16.0 ± 0.2[a]
Unsaturated				
C16:1 n-7	0.43 ± 0.04	1.71 ± 0.04[b]	0.34 ± 0.07	2.07 ± 0.23[b]
C18:1 n-9	9.47 ± 0.36	19.0 ± 0.1[b]	15.5 ± 0.3	16.9 ± 0.4[a]
C18:1 n-7	2.68 ± 0.37	1.72 ± 0.11[a]	4.86 ± 0.60	3.02 ± 0.15[a]
C18:2 n-6	8.43 ± 0.59	27.8 ± 0.3[b]	0.61 ± 0.06	1.67 ± 0.05[b]
C20:4 n-6	33.6 ± 0.48	15.5 ± 0.11[b]	11.9 ± 0.4	16.9 ± 0.4[b]
C20:3 n-3	2.37 ± 0.87	0.32 ± 0.06[a]	–	–
C22:4 n-6	–	–	2.87 ± 0.21	2.04 ± 0.07[a]
C22:5 n-6	–	–	0.62 ± 0.06	1.64 ± 0.08[b]
C22:6 n-3	2.87 ± 0.05	0.90 ± 0.02[b]	19.7 ± 0.4	11.5 ± 0.1[b]
Mono	13.7 ± 0.7	23.6 ± 0.1[b]	22.2 ± 1.0	22.9 ± 0.5
Σ n-9	10.1 ± 0.3	20.4 ± 0.2[b]	15.9 ± 0.4	17.9 ± 0.4[a]
Σ n-7	3.98 ± 0.50	3.96 ± 0.10	6.4 ± 0.7	5.64 ± 0.25
Σ n-6	43.4 ± 0.2	45.1 ± 0.2[b]	16.2 ± 0.29	22.7 ± 0.5[b]
Σ n-3	6.73 ± 0.64	3.51 ± 0.08[b]	20.19 ± 0.52	12.5 ± 0.1[b]
n-6/n-3	6.78 ± 0.69	12.9 ± 0.3[b]	0.80 ± 0.03	1.82 ± 0.04[b]
%Unsaturates	65.1 ± 1.1	73.0 ± 0.2[b]	59.9 ± 1.3	58.8 ± 0.9
[c]Unsaturation index	202 ± 3	166 ± 1[b]	207 ± 3	187 ± 3[b]
Average length	18.4 ± 0.1	18.1 ± 0.0	18.5 ± 0.0	18.4 ± 0.0
Cholesterol ($\mu g \cdot mg\ Protein^{-1}$)	56.2 ± 0.5	69.9 ± 2.2[a]	116.0 ± 1.0	136.3 ± 1.0[b]

[a] and [b] Indicate difference between the species at $P < 0.05$ and $P < 0.001$ respectively
[c] Unsaturation index is average number of double bonds per fatty acid residue × 100

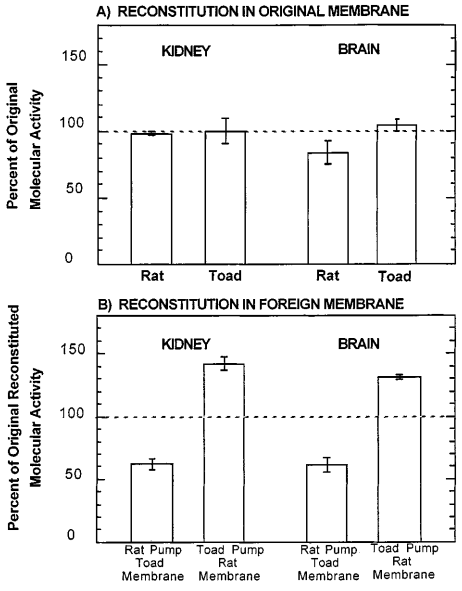

Fig. 2 **A** Percent recovery of sodium pump molecular activity of detergent-treated kidney and brain microsomes from *R. norvegicus* and *B. marinus* reconstituted into their own original microsomal membrane. Microsomal membranes (containing active sodium pumps) were reconstituted after detergent treatment that reduced activity to 8–15% of original maximal activity in kidney and brain respectively. Heat-treated microsomes (no sodium pump activity but unchanged phospholipid fatty acid composition) from the same organ were used to reconstitute activity. Results are expressed as a percentage of original molecular activity (see Table 1 for values) and show most of the original activity can be recovered in reconstitution experiments (see Materials and methods). Delipidation detergent concentration levels used in both original and foreign membrane reconstitutions in mg DOC:mg protein were, for brain, 4.0 for rat and 4.5 for toad, and for kidney they ranged from 3.5–5 for rat and 3.5–7 for toad. Detergent concentrations used were determined from the pattern of activity in response to increasing detergent concentrations of each individual microsomal preparation. **B** Percent recovery of sodium pump molecular activity in detergent treated kidney and brain microsomes of *R. norvegicus* and *B. marinus* reconstituted using microsomal membrane from the alternate species. *R. norvegicus* microsomes (with active sodium pumps) reconstituted with heat-treated *B. marinus* microsomes and *B. marinus* microsomes (with active sodium pumps) reconstituted with *R. norvegicus* heat-treated microsomes. Reconstituted molecular activities are expressed as a percentage of control reconstitutions (shown above) using original microsomal membrane. The results show high molecular activity associated with sodium pumps reconstituted into heat-treated rat microsomal membrane and low activity with reconstitutions using heat-treated toad microsomal membrane. Each preparation used the combined microsomal membranes isolated from 10–20 organs. Eight preparations were performed for kidney and from two to four for brain. Error bars are SEMs

sequent reduction in detergent concentration allowed sodium pumps to almost fully reconstitute their original activity. However, control reconstitutions carried out with equivalent amounts of buffer only resulted in the recovery of half of the original molecular activity (52–58 ± 1–8%) in all preparations. Residual lipids not removed during the delipidation could account for this activity. This result also indicates that additional membrane lipid (i.e. added microsomal membrane) is a requirement for recovery of high levels of molecular activity. The ability of rat membrane to improve the activity of toad sodium pumps may also be due to the presence of lipids that reconstitute more easily than those present in toad membrane. If so, this suggests that rat membrane must possess more of these different lipids or have easier access to these lipids than toad membrane, indicating intrinsic membrane differences between the species.

In order to remove further lipid from around the sodium pump to improve foreign lipid incorporation, further detergent treatment of the microsomes would be required. However, our experiments indicate that if molecular activities fall much below 200 $ATP \cdot min^{-1}$, sodium pumps may no longer reconstitute with increased activity. This suggests an upper limit to detergent treatment and lipid dispersal and presumably a minimum lipid requirement in order to maintain the functional integrity of the sodium pump protein.

Microsomes were used in these experiments as they provided a membrane system close to intact tissue compared to artificial membranes, in terms of composition and structure plus the large quantity of membrane required to perform these experiments. Retention of the difference in molecular activity between the rat and toad organs using microsomal membranes is not surprising. The molecular activity of mammalian sodium pumps has been found to be similar in preparations ranging from intact tissue to purified membranes (see Table 3 in Else et al. 1996 for a review). Similarly, the molecular activity of toad sodium pumps would not be expected to change simply because of the preparation used. This fact tends to confirm the potential importance of membranes in determining molecular activity.

The molecular activity values for rat kidney and brain in the present study are almost identical to those previously found in other studies for the same tissues (Jørgensen 1974; Sweadner 1989). What is different to other studies are the *B. marinus* values and those for other ectotherms we have previously reported (Else et al. 1996). In contrast to our general findings, high molecular activities have been reported for a number of ectothermic organs (Bader et al. 1968; Skou and Esmann 1979). In many of these cases, the organs used are associated with salt transport, i.e. salt glands and gills. However, there are exceptions, such as frog brain (Bader et al. 1968). It would be interesting to examine the membranes of these organs and to compare them with those of mammals to see if common characteristics exist.

An alternative explanation for differences in molecular activity differences between endotherms and ectotherms is differences in the sodium pump itself. Other than the ancestral $\alpha 1$ isoform, the types of sodium pumps present in the various organs of ectotherms are not well known (Horisberger 1994; Sweadner 1989). In contrast, at least three different α (the major catalytic subunit) and β isoforms are known, and evidence for other isoforms in the tissues of mammals and birds exists (Horisberger 1994). Therefore the possibility of different

isozymes determining sodium pump molecular activities exists. However, in mammals, sodium pumps with high molecular activities in kidney and heart are dominated by the same ancestral α1 isoform with low molecular activities (Else et al. 1996) known to be present in organs of reptiles, amphibians and fish (Sweadner 1989). Likewise, within mammals, different organs with different isozymal composition (Sweadner 1989) do not differ greatly in their molecular activity (Else et al. 1996). Therefore present evidence suggests that different isoforms do not account for the difference in molecular activity between endotherms and ectotherms. It seems likely that these isoforms are more important in their currently accepted roles such as sensitivity to endogenous inhibitors, Na^+/K^+ affinities and ability to regulate expression pathways (Horisberger 1994).

Based on the recognised lipid requirement for membrane-bound enzymes to express maximal activity (Anner 1985; Cornelius 1991; Kimelberg and Papahadjopoulos 1972; Stubbs and Smith 1984), and previous work suggesting characteristic membrane fatty acid composition differences between endotherms and ectotherms (Hulbert 1993), lipids and their constituent fatty acids may be part of the membrane effect on molecular activity of the sodium pump (see Table 2). Membrane proteins are less likely to have caused the changes measured in the present experiments due to the prior heat treatment of the added microsomal membrane.

The improvement in performance of toad sodium pumps occurred under less than ideal conditions where some residual lipid presumably remains to maintain the integrity of the protein. Yet even under these conditions, an increase in molecular activity of up to 40% is possible and in the case of the rat a 40% reduction in activity occurred. Under ideal conditions in which toad sodium pumps existed within an increased rat membrane lipid environment, molecular activities may be expected to increase further. Likewise, rat sodium pumps within an increased toad membrane lipid environment would be expected to show further decreases in activity. While this work used only two species and two organs it points to the potential for different membrane characteristics to produce very different molecular activities.

This work has indicated that membrane composition may be a contributing factor to the molecular activity difference found between endotherms and ectotherms. Whether lipid, protein or other membrane-associated molecules turn out to be the factor involved remains to be resolved. Based on the general results presented in this current work, our next set of experiments will be aimed at examining lipid involvement in sodium pump activity. Specifically, we will fuse vesicles prepared from the natural phospholipids of each species with microsomal membranes to direct lipid compositional changes while determining molecular activity.

The work presented here has potentially broad implications. For example, dietary fat profiles are known to change membrane phospholipid fatty acid composition (Charnock 1994; Knapp et al. 1994; Rajotte et al. 1988; Zakim 1986). If lipids are found to be critical in determining molecular activities, in humans who ingest energy primarily derived from saturated fat, small changes in membrane composition may have subtle effects on the performance of membrane proteins such as the sodium pump.

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