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An allometric comparison of microsomal membrane lipid composition and sodium pump molecular activity in the brain of mammals and birds

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Summary

Previous research has shown that the lipid milieu surrounding membrane proteins may be an important factor in determining their activity. To investigate this we have examined sodium pump molecular activity and microsomal membrane lipid composition in the brain of five mammalian and eight avian species ranging in size from 30 g mice to 280 kg cattle and 13 g zebra finches to 35 kg emus, respectively. Sodium pump (Na^+,K^+-ATPase) activity was higher in the smaller species and showed a significant allometric decline with body mass in both the mammals (μmol P_i h^{-1} mg wet $mass^{-1} = 6.2\times mass^{-0.06}$) and birds ($\mu$mol P_i h^{-1} mg wet $mass^{-1} = 5.4\times mass^{-0.07}$). In small mammals, the elevated enzyme activity was related to allometric changes in both the concentration and the molecular activity (turnover rate) of sodium pumps, while in birds, no significant body-size-related variation was observed for either sodium pump concentration or molecular activity. Microsomal phospholipid fatty acid profile displayed little allometric variation in both the mammals and birds and was not correlated with molecular activity in either group. Brain phospholipids from both endothermic classes were dominated by the long chain *n*-3 polyunsaturate, docosahexaenoic acid [22:6 (*n*-3)], which accounted for an average of 28% and 34% of the total fatty acids in the mammals and birds respectively. Bird membranes also contained a relatively large percentage of 22:5 (*n*-6) as well as high levels of cholesterol. These results are discussed in relation to neurological function and the emerging field of membrane lipid rafts.

Key words: body size, cholesterol, docosahexaenoic acid, fatty acids, Na^+,K^+-ATPase, phospholipids.

Introduction

Metabolic rate varies substantially between vertebrate species, in particular being much greater on a mass-specific basis in small mammals and birds than their larger counterparts (Kleiber, 1961; Lasiewski and Dawson, 1967). While some of this difference in metabolic activity is related to the size of internal organs, much of the difference relates to the activity of processes at the cellular level. For example, the respiration rate of tissue slices is higher in small mammals compared to large mammals (Couture and Hulbert, 1995b), with the same allometric trends also seen in isolated hepatocytes in mammals (Porter and Brand, 1995) and birds (Else et al., 2004).

At the cellular level, a substantial proportion of metabolic rate is associated with the maintenance of ion gradients across membranes (Rolfe and Brown, 1997), with one of the most important gradients being the Na^+ gradient. The sodium pump (Na^+,K^+-ATPase), a ubiquitous enzyme found in the cell membrane of all animals, is responsible for the maintenance of this Na^+ gradient. In humans and rats, the *in vivo* activity of the sodium pump is estimated to account for approximately 20% of resting metabolic rate (Rolfe and Brown, 1997), however in tissues such as the kidney and brain, where sodium pump concentration is the highest, it can account for as much as 60% of resting metabolism (Clausen et al., 1991).

The *in vitro* activity of the sodium pump varies considerably between species. For example, in addition to measuring the respiration rate of mammalian liver and kidney slices, Couture and Hulbert (1995b) also determined sodium pump activity (measured as $[^{86}Rb]^+$ uptake), and found that activity was higher in small mammals and declined with allometric exponents of –0.13 and –0.14 in the liver and kidney respectively. This study however, did not determine whether tissues in small mammals had an increased sodium pump activity due to a greater concentration of sodium pumps or an increased molecular activity of individual sodium pumps (i.e. turnover rate of substrate per enzyme). Comparisons of endotherms and ectotherms have shown that the higher metabolic rate of the laboratory rat (*Rattus norvegicus*) compared to the cane toad (*Bufo marinus*) is associated with a 3–4-fold greater sodium pump molecular activity in rat tissues (Else et al., 1996; Else and Wu, 1999), and it has been proposed

that changes in the molecular activity of membrane proteins are one of the major mechanisms underlying differences in metabolism (Hulbert and Else, 1999). Thus, the first aim of the current study was to determine sodium pump molecular activity in the brain of mammals and birds of different body size, to examine if the high mass-specific metabolic rate of small endotherms is associated with an increased molecular activity in their sodium pumps.

In the second part of the present investigation we have examined microsomal membrane lipid composition in the brain of the mammalian and avian species. Changes in the fatty acid composition of neural membranes have been shown to alter the kinetic properties of the sodium pump (Gerbi et al., 1993, 1994), and recently functional reconstitution experiments involving lipid crossovers between species, have provided direct evidence that membrane fatty acid composition is a major determinant of the molecular activity of the sodium pump enzyme (Else and Wu, 1999; Wu et al., 2004). Accordingly, we have analysed membrane lipid composition to determine whether variations in sodium pump molecular activity in the brain of the mammals and birds are underpinned by variations in membrane fatty acid composition.

Materials and methods

Animals

The mammals and birds examined in the present study were adults of either sex (see Table 1). Mice (*Mus musculus* L.) and rats (*Rattus norvegicus* Berkenhout) were purchased from Gore Hill Research Laboratories (Sydney, NSW, Australia) and housed in the University of Wollongong animal house at 22±2°C with 12:12 light:dark photoperiod and *ad libitum* access to food (rodent pellets) and water. Mice were killed by cervical dislocation, while rats were killed by Nembutal® overdose (pentobarbitone sodium, 100 mg kg^{-1} body mass; intraperitoneal injection). Tissues from sheep (*Ovis aries* L.), pigs (*Sus scrofa* L.) and cattle (*Bos taurus* L.) were obtained from a local abattoir (Wollondilly, NSW, Australia), where immediately following death the tissues were transported on ice back to the University for use in the experiments. The diet of the sheep, pigs and cattle before death was unknown.

Emus (*Dromaius novaehollandiae* Latham) were purchased from Marayong Park Emu Farm (Falls Creek, NSW, Australia). Zebra finches (*Taeniopygia guttata* Vieillot), ducks (*Anas platyrhynchos* L.) and geese (*Anser anser* L.) were purchased from local pet shops or at the Narellan Aviary Bird Auction (NSW, Australia). Feral pigeons (rock dove, *Columba livia* Gmelin) were from a local pigeon breeder (T. Cooper, Corrimal, NSW, Australia). House sparrows (*Passer domesticus* L.), starlings (*Sturnus vulgaris* L.), and pied currawongs (*Strepera graculina* Shaw) were free-living animals caught locally in the Wollongong area. Birds were either used immediately on the day of collection or were housed short-term (2–3 days) in the University of Wollongong animal house under the same environmental conditions as described for mammals, with *ad libitum* access to food and water. For the finches and sparrows the food was mixed birdseed, and for the ducks and geese it was a commercial mixture of pellets and seeds. The diet of the other birds before purchase was unknown. All birds were killed by lethal overdose of either Lethabarb® or Nembutal® (pentobarbitone sodium, 100 mg kg^{-1} body mass; intraperitoneal, except in the case of the emus where the injection was intrajugular).

Body mass and brain mass of the mice, rats and all the bird species were obtained immediately following death. For sheep and cattle, carcass weights were used to calculate the weight of the whole mammal, assuming carcass weight was 55% of total body mass as is routinely used commercially (Couture and Hulbert, 1995b). The pig carcass weight included the skin, and since this organ accounts for 15–20% of total body mass, carcass weight was assumed to be 70% of total body mass. Brain mass for the sheep, pigs and cattle were obtained immediately prior to the commencement of experimental assays. All procedures were performed in accordance with the National Health and Medical Research Council Guidelines for Animal Research and were approved by the Animal Experimentation Ethics committee of the University of Wollongong.

Materials

[^{3}H]ouabain (30.0 Ci $mmol^{-1}$, 37 MBq, 96.2% purity) in 1:9 toluene:ethanol was obtained from Amersham Pharmacia Biotech (Castle Hill, NSW, Australia). Ouabain was purchased from ICN Biomedicals Inc. (OH, USA), scintillation cocktail (Ready Safe™) from Beckman (Gladesville, NSW, Australia), tissue solubiliser (Soluene®-350) from Packard Biosciences (Mt Waverley, VIC, Australia) and Na_2ATP (special quality) from Boehringer Mannheim (Mannheim, Germany). Sodium deoxycholate (DOC), ethylenediaminetetracetic acid (EDTA), ammonium molybdate, ferrous sulphate, analytical grade methanol, chloroform, ethyl acetate, n-hexane, and extra pure grade diethyl ether and petroleum spirit (40–60°C) were purchased from Merck Pty Ltd (Kilsyth, VIC, Australia). Lethabarb® and Nembutal® (pentobarbitone sodium) were from Boehringer Ingelheim Pty Ltd (Artarmon, NSW, Australia). Analytical grade butylated hydroxytoluene (BHT) 14% (w/v), boron trifluoride in methanol, stannous chloride, lab reagent sodium hydrosulfite (~80%), the cholesterol assay kit, the cholesterol calibrator and all fatty acid standards were from Sigma Aldrich (Castle Hill, NSW, Australia). Silane-treated glass wool was purchased from Alltech associates (Baulkham Hills, NSW, Australia). Strata® SPE SI-2 Silica and FL-PR Florisil columns were from Phenomenex (Pennant Hills, NSW, Australia). All other chemicals and reagents used were of analytical grade and obtained from Ajax chemicals (Auburn, NSW, Australia).

[^{3}H]ouabain binding

The concentration of sodium pump sites was determined using the [^{3}H]ouabain binding method described by Else et al. (1996). Brains were removed from the animals immediately following death, cortical sections were cut into small pieces

(2–10 mg) and placed in ice-cold K^+-free medium that closely resembled the ionic composition of mammalian and avian plasma (see below). Mammalian K^+-free medium was based on a solution previously used (Else at al., 1996) and included (in mmol l^{-1}): 125 NaCl, 1.2 $MgSO_4$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 1.3 $CaCl_2$ and 5 glucose, pH 7.4. As [^{3}H]ouabain binding had not previously been conducted in avian brains, two different solutions were used to assess [^{3}H]ouabain binding in the birds (in mmol l^{-1}): a K^+-free medium (124 NaCl, 1.2 $MgSO_4$, 1.1 NaH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$ and 11.1 glucose, pH 7.4), and a 4.5 mmol K^+-medium (125 NaCl, 3.4 KCl, 1.2 $MgSO_4$, 1.1 KH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$ and 11.1 glucose, pH 7.4). A K^+ concentration of 4.5 mmol was chosen for the incubations as it approximates the documented average plasma K^+ concentration for a large number of birds (Altman and Dittmer, 1974; Prosser, 1973). K^+-free media are generally used in [^{3}H]ouabain binding studies as K^+ is thought to inhibit binding of ouabain to the sodium pump (Wallick et al., 1980); however, Else (1994) demonstrated increased levels of binding with varying levels of K^+. Under the current experimental conditions, there was no statistical difference in the measured [^{3}H]ouabain binding sites using the different media, and therefore their average was used to estimate sodium pump density in birds.

Brain tissue samples were preincubated in ice-cold K^+-free medium for 2×10 min periods to reduce tissue K^+. The samples were then incubated in 2 ml of mammalian or avian K^+-free medium containing 1 μCi ml^{-1} [^{3}H]ouabain and a final ouabain concentration of 5×10^{-5} mol l^{-1}. Parallel incubations containing the same amount of [^{3}H]ouabain and a final concentration of 10^{-2} mol l^{-1} ouabain were also conducted to determine non-specific binding. Mammalian tissues were assayed at 37°C, while avian incubations were completed at 40°C. Incubations were gassed continuously for 2 h with carbogen (5% CO_2, 95% O_2), to maintain physiological pH levels (7.4), and to circulate the incubation medium around the tissue samples.

After incubation, the tissue samples were washed five times (8 min per wash) in 3 ml of ice-cold K^+-free medium to reduce [^{3}H] activity associated with non-specific sites, as previously characterised (Else, 1994; Else et al., 1996). Following the wash procedure, samples were blotted lightly, weighed (±0.01 mg) and placed in 200 μl of tissue solubiliser (Soluene®-350) overnight. Readysafe scintillation cocktail (2 ml) was added to each vial and [^{3}H] activity counted on a Wallac 1409 Liquid Scintillation Counter (Turku, Finland) with d.p.m. correction.

[^{3}H]ouabain binding was expressed as relative uptake, i.e. [^{3}H] activity taken up per gram wet weight of tissue relative to [^{3}H] activity in the incubation medium. Specific uptake was calculated following subtraction of [^{3}H] activity determined in excess ouabain (10^{-2} mol l^{-1}), which was deemed non-specific uptake. [^{3}H]ouabain binding sites per gram of tissue were determined by multiplying the specific uptake by the total ouabain concentration in the medium. Sodium pump density was calculated assuming a 1:1 stoichiometry between sodium pump units and ouabain binding sites and was expressed as picomoles of sodium pumps per gram of brain wet mass.

Determination of Na^+,K^+-ATPase activity

Na^+,K^+-ATPase activity was determined in brain homogenates using a modified method of that described by Esmann and Skou (1988). Dilute homogenates were prepared (2%, w/v) in ice-cold 250 mmol l^{-1} sucrose, 5 mmol l^{-1} EDTA,

Table 1. *Body mass, brain mass, brain protein concentration and basal metabolic rate of the mammals and birds*

Common name	Scientific name	Body mass (g)	Brain mass (g)	Brain protein concentration (mg g^{-1} wet mass)	BMR* (kcal g^{-1} day^{-1})
Mammals					
Mouse	*Mus musculus*	37.9±1.1 (16)	0.47±0.01 (16)	152±6 (16)	0.171
Rat	*Rattus norvegicus*	281±6 (12)	1.78±0.03 (12)	148±4 (12)	0.100
Sheep	*Ovis aries*	38500±1380 (8)	96±4 (8)	138±3 (8)	0.028
Pig	*Sus scrofa*	88300±6990 (8)	81±3 (4)	131±4 (8)	0.019
Cow	*Bos taurus*	277000±20800 (8)	311±17 (4)	126±5 (8)	0.015
Birds					
Zebra finch	*Taeniopygia guttata*	12.6±0.9 (4)	0.42±0.01 (4)	138±2 (4)	0.370
Sparrow	*Passer domesticus*	25.9±0.9 (4)	0.86±0.03 (4)	149±2 (4)	0.278
Starling	*Sturnus vulgaris*	75±3 (4)	1.69±0.06 (4)	149±3 (4)	0.260
Currawong	*Strepera graculina*	283±19 (4)	5.38±0.15 (4)	144±8 (4)	0.179
Pigeons	*Columba livia*	462±35 (4)	2.11±0.05 (4)	120±10 (4)	0.075
Duck	*Anas platyrhynchos*	2178±61 (4)	6.12±0.21 (4)	127±8 (4)	0.071
Goose	*Anser anser*	4444±360 (4)	10.9±0.17 (4)	130±4 (4)	0.062
Emu	*Dromaius novaehollandiae*	34975±745 (4)	20.5±0.87 (4)	115±4 (4)	0.017

Values are means ± S.E.M. (number of animals measured in parentheses).

*BMR values for mammals are from Kleiber (1961), while BMR values for birds are from Hulbert et al. (2002a), and were converted from ml O_2 g^{-1} h^{-1} to kcal g^{-1} h^{-1} using a conversion factor of 4.7, which assumes an RQ of 0.7.

Table 2. *Na^+,K^+-ATPase activity, sodium pump density, and molecular activity of sodium pumps from the brain of mammals and birds*

	Na^+,K^+-ATPase activity (μmol P_i h^{-1} mg^{-1} wet mass)	Sodium pump density (pmol g^{-1} wet mass)	Molecular activity (ATP min^{-1})
Mammals			
Mouse	4.70±0.26 (9)	3180±407 (9)	28852±4396 (9)
Rat	5.01±0.23 (3)	3093±452 (3)	27775±2824 (3)
Sheep	2.99±0.19 (4)	5580±488 (4)	9167±1136 (4)
Pig	2.72±0.22 (4)	4192±1007 (4)	12914±2924 (4)
Cow	3.40±0.18 (4)	5002±321 (4)	11372±514 (4)
Birds			
Zebra finch	4.57±0.15 (4)	5262±984 (4)	15848±2551 (4)
Sparrow	3.80±0.31 (4)	3251±430 (4)	21537±5164 (4)
Starling	3.77±0.15 (4)	7498±995 (4)	8759±1040 (4)
Currawong	3.95±0.31 (4)	3841±313 (4)	17502±1873 (4)
Pigeon	3.27±0.12 (4)	6720±1476 (4)	9435±2077 (4)
Duck	3.60±0.28 (4)	4437±761 (4)	15145±3386 (4)
Goose	3.04±0.17 (4)	3559±860 (3)	16559±3426 (3)
Emu	2.17±0.10 (4)	2627±287 (4)	14357±1842 (4)

Values are means ± S.E.M. (number of preparations in parentheses). Na^+,K^+-ATPase activity measurements were conducted at 37°C in mammals and are corrected to 37°C in birds (see Materials and methods section). Molecular activity is maximal Na^+,K^+-ATPase activity divided by the sodium pump density for the same animal. P_i, inorganic phosphate.

20 mmol l^{-1} imidazole (pH 7.4) using a glass–glass homogeniser. A mild detergent treatment was applied to the samples prior to the assay to elicit maximal Na^+,K^+-ATPase activity. A 150 µl volume of homogenate was mixed under constant stirring with 150 µl of sodium deoxycholate (1 mg ml^{-1}) and was allowed to stand at room temperature for 15 min. Samples (50 µl) of the detergent treated homogenates were then preincubated in Na^+,K^+-ATPase assay medium (in mmol l^{-1}: 30 histidine, 4 $MgCl_2$, 124 NaCl, and either 1 ouabain or 20 KCl, pH 7.5) for 10 min at 37°C (mammals) or 40°C (birds) to allow for thermal equilibration and binding of ouabain to the sodium pumps. Enzyme activity was initiated by the addition of 3 mmol l^{-1} ATP and allowed to proceed for 5 min. The reaction was terminated by the addition of an equal volume of perchloric acid (0.8 mol l^{-1}) at 4°C. Inorganic phosphate (Pi) was determined as previously described (Else, 1994). Maximal Na^+,K^+-ATPase activity was calculated as the difference in inorganic phosphate liberated (from ATP) in the presence and absence of 1 mmol l^{-1} ouabain (minus and plus KCl, respectively). Experiments were conducted in duplicate or better.

Mammals and birds were assayed at different temperatures to approximate *in vivo* conditions. To allow comparison between the two vertebrate classes, thermal quotient (Q_{10}) values for Na^+,K^+-ATPase activity from the brain of several birds was determined. These Q_{10} values were all close to 2.0 (range 1.6–2.4), and as such a this value was used to correct bird Na^+,K^+-ATPase activity to 37°C and therefore allow comparison with mammals.

Molecular activity

Molecular activity is defined as the maximal rate of substrate turnover by a protein, and for the sodium pump was derived by dividing maximal Na^+,K^+-ATPase activity (expressed as pmol P_i min^{-1} mg wet $mass^{-1}$) by the number of sodium pumps (in pmol mg wet $mass^{-1}$) for the same preparation. The net result was expressed as the number of ATP molecules hydrolysed by each sodium pump per minute.

Preparation of microsomal membranes

All lipid measurements were conducted using microsomal membranes prepared from brain (cortex) homogenates (10% in 250 mmol l^{-1} sucrose, 20 mmol l^{-1} imidazole, 1 mmol l^{-1} EDTA; pH 7.4) that were centrifuged at 3000 ***g*** for 3 min and a further 10 min at 10,000 ***g*** to remove nuclei and mitochondria respectively. The supernatant was then centrifuged at 98,000 ***g*** for 35 min and the resultant pellet, designated microsomal membranes, was resuspended in 25 mmol l^{-1} imidazole, 2 mmol l^{-1} EDTA (pH 7.5). In the current investigation we examined microsomal membranes in preference to whole tissue, as phospholipids isolated from microsomes (i.e. plasma membrane, golgi and endoplasmic reticulum phospholipids) are more representative of the lipids that would be directly surrounding the sodium pump, rather than whole tissue phospholipids which would also contain nuclear and mitochondrial membrane fractions. Furthermore we prepared microsomes for all species from the same area of the brain where sodium pump determinations were conducted (i.e. cerebral cortex), to minimise any region-related differences and to allow direct comparison between the sodium pump and membrane lipid measurements. Microsomal fractions were prepared from the tissue of individual animals, except for the mouse and zebra finch where some microsomal fractions were pooled samples from the tissue of 2–3 animals. The protein content of

microsomal preparations (and tissue homogenates) was determined by the Lowry method, using bovine serum albumin as the standard.

Analysis of phospholipid fatty acid composition

Total lipid was extracted from the microsomal preparation by standard methods (Folch et al., 1957) using ultra-pure grade chloroform:methanol (2:1, v/v) containing butylated hydroxytoluene (0.01%, w/v) as an antioxidant. Phospholipids were separated from neutral lipids by solid phase extraction on silicic acid columns. Fatty acid analysis of the phospholipid fraction was determined as described in detail elsewhere (Pan and Storlien, 1993). The cholesterol content of microsomal preparations was determined by enzymatic assay (Sigma chemicals). Analysis of phospholipid content was via a phosphorus assay (Mills et al., 1984).

Statistical analyses

All statistical comparisons were determined and tested for significance using the mean value for each species (i.e. N=5 for mammals and N=8 for birds). Allometric equations were determined by linear regression (least-square method) of log-transformed values using JMP® 4.0.1 software (SAS Institute Inc., NC, USA). All figures were produced using KaleidaGraph™ 3.51 software (Synergy Software, Reading, PA, USA). Allometric relationships were tested for significance using the Pearson product moment correlation coefficient, with n-2 degrees of freedom. Significance for all relationships was accepted at the level of P<0.05 and all results are reported as means ± standard error of the mean (S.E.M.).

Results

Table 1 presents the body mass and mass-specific basal metabolic rate (BMR) of the mammals and birds examined in the current study. The species were chosen to span as wide a range of body mass as practical, and on the basis of availability. Mass-specific BMR was lower in the larger species with mammalian BMR (kcal g^{-1} day^{-1}) = 0.47×mass $(g)^{-0.27}$ (P<0.001) and avian BMR (kcal g^{-1} day^{-1}) = 1.06× mass $(g)^{-0.37}$ (P<0.001). The allometric exponent describing mammalian BMR in the current study is close to the value of –0.25 that generally approximates the BMR exponent in mammals (Withers, 1992), while the allometric exponent observed in the birds (–0.37) is very steep and is the result of the fact that the four smallest birds were passerines, which generally possess higher rates of basal metabolism than non-passerines, which comprised the four larger species (Lasiewski and Dawson, 1967).

Smaller species had relatively larger brains, particularly in the birds where the brain represented 3.3% of body mass in the zebra finch, but only 0.05% in the emu (Table 1). The allometric exponents describing the body-size-related variation in brain mass were 0.72 and 0.47 for mammals and birds respectively. Protein concentration (per gram of wet mass) was significantly higher in the smaller species, declining with

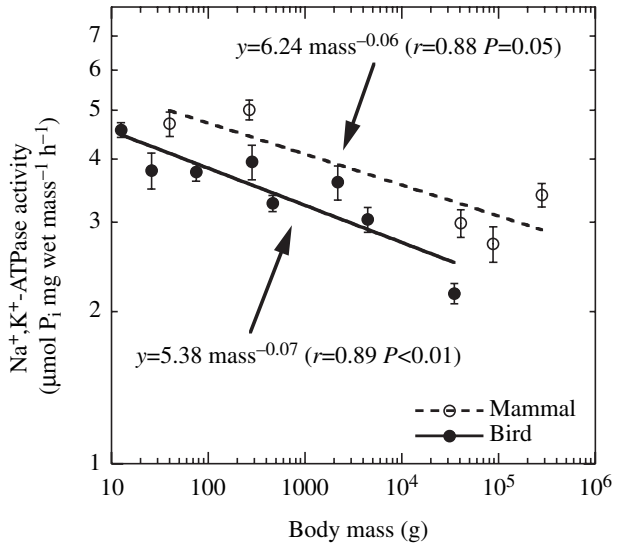

Fig. 1. Allometric relationship between body mass and Na^+,·K^+-ATPase activity of brain homogenates in mammals and birds. Values are means ± S.E.M. of Na^+,·K^+-ATPase measurements at 37°C in mammals and corrected to 37°C in birds (see Materials and methods). Data from Tables 1 and 2. The lines are the best power fits to the data as described by the inset equations. P_i, inorganic phosphate.

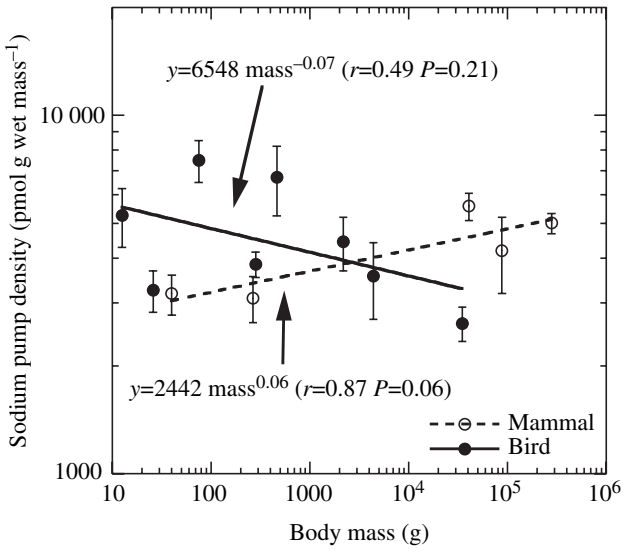

Fig. 2. Allometric relationship between body mass and brain sodium pump density in mammals and birds. Values are means ± S.E.M. Data from Tables 1 and 2. The lines are the best power fits to the data as described by the inset equations.

allometric exponents of –0.02 and –0.03 in the mammals and birds respectively. As such the brains of the mice contained 17% more protein (per gram of wet mass) than those of cattle, while sparrow and starling brains contained 23% more protein than emu brains (Table 1).

Na^+,K^+-ATPase activity, sodium pump density, and molecular activity measured for the mammals and birds are presented in Table 2. Na^+,K^+-ATPase activity values were measured at 37°C for mammals and corrected to 37°C for birds

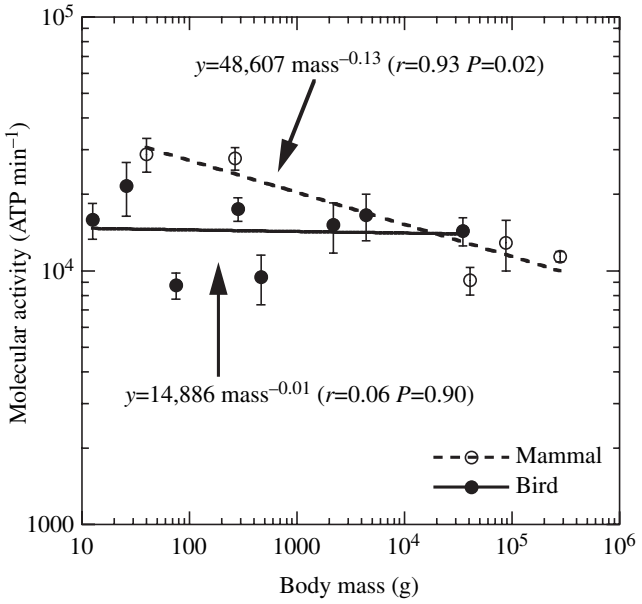

Fig. 3. Allometric relationship between body mass and sodium pump molecular activity in the brain of mammals and birds. Values are means ± S.E.M. Molecular activity values were calculated by dividing maximal Na^+,·K^+-ATPase activity at 37°C by the sodium pump density for the same animal. Data from Tables 1 and 2. The lines are best power fits to the data as described by the inset equations.

(see Materials and methods). When examined relative to body mass, there was a significant decrease in Na^+,K^+-ATPase activity for both the mammals (P=0.05) and birds (P<0.01) (Fig. 1). Calculated from the allometric exponents (–0.06 and –0.07), for every doubling in body mass there would be a 4.1% and 4.7% decrease in Na^+,K^+-ATPase activity in the mammals and birds respectively.

Sodium pump density per gram of brain wet mass was generally similar between the mammal and bird species. When considered relative to body mass (Fig. 2), there was a close to significant (P=0.056) allometric increase in sodium pump density in the larger mammals, with an exponent of 0.06 observed. In the brain of birds the trend was for a greater sodium pump density in the smaller species, although the exponent describing this relationship (–0.07) was not statistically different from zero.

Molecular activity was calculated by dividing maximal Na^+,K^+-ATPase activity (at 37°C) by the sodium pump density to give the number of ATP molecules hydrolysed by each sodium pump per minute (ATP min^{-1}). In mammals, molecular activity varied approximately threefold over the body mass range from 9000–29,000 ATP min^{-1}, while for birds less variation was seen, with most species having a molecular activity of approximately 15,000 ATP min^{-1} (Table 2). When examined relative to body mass (Fig. 3) there was a significant (P=0.02) decrease in molecular activity in the mammals with body mass explaining 86% of the variability. In contrast with this, less than 1% of the variability in bird brain molecular activity values could be explained by body mass (Fig. 3).

Table 3 presents the cholesterol and phospholipid content, along with their molar ratio, in brain microsomal membranes from the mammals and birds. No significant body-size-related variation was observed in any of the parameters presented in Table 3. Comparison of the mammals and birds shows that despite a similar microsomal cholesterol content (per mg of protein), birds have a greater molar ratio of cholesterol:phospholipid in their microsomal membranes, due to a lower content of phospholipid. From the cholesterol:phospholipid ratios it can be seen that brain microsomes from mammals would contain approximately 2–3 phospholipids per molecule of cholesterol, while in brain

Table 3. *Cholesterol and phospholipid content of microsomal membranes from the brain of mammals and birds*

	Cholesterol (μg mg^{-1} protein)	Phospholipid (μg mg^{-1} protein)	Cholesterol:phospholipid (mol:mol)*
Mammals			
Mouse (4)	72.9±3.4	271±25	0.55±0.05
Rat (3)	93.0±9.3	405±27	0.47±0.06
Sheep (4)	102±7.1	388±26	0.53±0.03
Pig (4)	107±5.8	575±51	0.38±0.02
Cow (4)	91.0±2.5	470±16	0.39±0.01
Birds			
Zebra finch (3)	84.4±10.7	227±42	0.78±0.14
Sparrow (4)	87.4±3.8	167±8	1.06±0.05
Starling (4)	102±6.7	264±32	0.80±0.08
Currawong (4)	96.4±4.5	310±17	0.63±0.04
Pigeon (4)	95.2±7.8	234±39	0.86±0.09
Duck (4)	92.8±2.8	238±3	0.79±0.03
Goose (4)	84.8±2.8	210±23	0.85±0.10
Emu (4)	83.3±5.3	281±62	0.65±0.08

Values are means ± S.E.M. Number of preparations used for each measurement in parentheses.
*Moles of phospholipid calculated assuming a molecular weight of 780.

Table 4. *Microsomal phospholipid fatty acid profiles from mammalian brains*

	Mouse	Rat	Sheep	Pig	Cow
Fatty acid					
16:0	18.1±0.6	17.1±1.7	17.0±0.3	16.9±0.5	14.1±0.7
18:0	14.1±0.9	14.6±1.8	15.1±0.5	16.5±0.5	12.8±1.5
16:1 (*n*-7)	0.3±0.0	0.3±0.0	0.5±0.1	0.6±0.0	0.5±0.1
18:1 (*n*-9)	13.3±0.2	14.7±0.4	14.6±0.6	19.0±2.1	12.2±1.5
18:1 (*n*-7)	4.6±0.5	4.0±0.3	4.5±0.2	6.3±0.3	3.9±0.3
18:2 (*n*-6)	1.8±1.3	0.8±0.1	0.5±0.0	0.8±0.1	0.5±0.1
20:1 (*n*-9)	1.0±0.0	1.6±0.1	0.3±0.0	0.6±0.1	0.2±0.1
20:2 (*n*-6)	1.3±0.9	0.3±0.2	0.8±0.1	0.5±0.2	0.5±0.2
20:3 (*n*-6)	0.4±0.0	0.4±0.1	0.6±0.0	0.5±0.0	0.6±0.0
20:4 (*n*-6)	11.9±0.5	13.1±0.7	8.6±0.3	11.9±0.2	9.3±0.6
22:4 (*n*-6)	3.2±0.2	4.5±0.2	3.6±0.2	5.5±0.2	0.9±0.4
22:5 (*n*-6)	0.5±0.0	0.8±0.1	0.6±0.0	3.0±0.4	0.9±0.1
22:5 (*n*-3)	0.2±0.0	0.3±0.1	2.2±0.2	0.7±0.0	1.8±0.1
22:6 (*n*-3)	28.6±1.5	27.0±2.5	30.0±1.6	16.5±2.1	37.5±4.1
% Saturates	32.7±1.3	32.1±3.5	32.8±0.8	33.8±0.8	27.6±3.3
% MUFA	19.5±0.6	20.8±0.7	20.0±0.8	26.6±2.5	17.1±1.9
% PUFA	47.8±0.8	47.2±3.1	47.2±1.5	39.5±2.3	55.3±5.2
% *n*-9	14.3±0.2	16.4±0.4	14.8±0.7	19.6±2.2	12.4±1.6
% *n*-7	5.2±0.5	4.4±0.3	5.2±0.2	7.1±0.3	4.7±0.4
% *n*-6	19.1±1.6	20.0±0.7	14.6±0.5	22.2±0.5	15.7±1.1
% *n*-3	28.8±1.5	27.2±2.4	32.6±1.8	17.4±2.0	39.6±4.2
% Unsaturates	67.3±1.3	67.9±3.5	67.2±0.8	66.2±0.8	72.4±3.3
Unsaturation index	262±6	262±18	269±9	219±11	314±27
Chain length	19.2±0.0	19.3±0.1	19.3±0.1	18.9±0.1	19.7±0.2
20+22C PUFA	47.1±0.9	48.0±3.3	46.9±1.3	39.2±2.3	55.0±5.1
n-6/*n*-3	0.7±0.1	0.7±0.0	0.5±0.0	1.3±0.1	0.4±0.0
20:4/18:2	19.3±5.8	16.5±2.2	16.2±0.8	14.8±2.2	22.5±3.3

Values are means ± S.E.M., N=4 for all preparations except the rat where N=3. Fatty acids are expressed as mol% of total fatty acids. Unsaturation index is the average number of double bonds per 100 fatty acid chains. Chain length is the average chain length of each fatty acid. Fatty acids where all species contained <0.5% are not included.

microsomes from birds there would be 1.0–1.5 phospholipids per molecule of cholesterol.

The fatty acid profile of brain microsomal phospholipids for mammals and birds are presented in Table 4 and Table 5 respectively. In phospholipids from mammalian brain microsomes, there were no statistically significant allometric trends observed for fatty acid composition. The brain of all mammals displayed a relatively high content of 22:6 (*n*-3), with the highest levels observed in the cattle and a considerably lower amount in the pigs (Table 4). On average 68% of the fatty acid chains were unsaturated, with an unsaturation index of 265. The mean values for the other major parameters were 20.8% monounsaturated fatty acids (MUFA), 47.4% polyunsaturated fatty acids (PUFA), 18.3% *n*-6 PUFA, 29.1% *n*-3 PUFA and 27.9% 22:6 (*n*-3).

In microsomal phospholipids from the avian brains there were a small number of significant allometric trends. The content of 18:2 (*n*-6) and 20:3 (*n*-6) were significantly lower (P<0.05) in the larger birds, which resulted in a significant allometric increase (P<0.01) in the ratio of 20:4 (*n*-6) : 18:2 (*n*-6), which is an estimate of Δ5 and Δ6 desaturase enzyme activity. All bird species had a high content of 22:6 (*n*-3) with the highest levels found in the emu and the lowest levels in the pigeon (Table 5). The emu and duck had the highest levels of unsaturation (as indicated by unsaturation index), which appeared to result from a greater content of 22:5 (*n*-6) in these species, plus the high levels of 22:6 (*n*-3). The average values for the major parameters were 71% total unsaturates, 314 unsaturation index, 13.2% MUFA, 58.0% PUFA, 23.1% *n*-6 PUFA, 34.9% *n*-3 PUFA, and 34.1% 22:6 (*n*-3).

Discussion

In the present investigation we have examined the relationship between body size, sodium pump molecular activity and microsomal membrane lipid composition, in the brain of mammals and birds. Sodium pump (Na^+,K^+-ATPase) enzyme activity was higher in the brain of smaller mammals and birds, showing a significant allometric decline in both groups (Fig. 1). These findings are in agreement with the allometric decline in sodium pump activity observed in liver and kidney slices of small mammals (Couture and Hulbert, 1995b), and suggest that higher mass-specific metabolic rate in

Table 5. *Microsomal phospholipid fatty acid profiles from avian brains*

	Zebra finch	Sparrow	Starling	Currawong	Pigeon	Duck	Goose	Emu
Fatty acid								
16:0	15.1±1.2	18.0±0.6	18.0±1.3	16.9±1.2	16.5±1.2	13.5±1.0	18.7±0.9	11.7±0.9
18:0	12.5±1.3	14.8±1.1	14.6±1.6	14.3±1.0	13.5±1.0	10.5±0.8	13.9±0.8	8.4±0.9
16:1(*n*-7)	0.5±0.0	0.4±0.0	0.4±0.0	0.6±0.0	0.6±0.0	0.4±0.0	0.7±0.1	0.7±0.0
18:1(*n*-9)	6.3±0.1	6.8±0.1	7.4±0.3	8.2±0.5	9.8±0.4	7.7±0.6	9.8±0.7	6.5±0.1
18:1(*n*-7)	4.9±0.1	6.2±0.2	5.6±0.2	4.4±0.3	6.2±0.4	3.5±0.1	4.0±0.3	3.7±0.2
18:2(*n*-6)	1.2±0.1	0.5±0.0	0.5±0.0	0.5±0.0	0.6±0.1	0.2±0.0	0.3±0.0	0.2±0.0
20:2 (*n*-6)	2.5±1.9	0.7±0.5	1.4±1.2	0.6±0.1	2.7±0.7	1.7±1.0	1.0±0.8	0.8±0.3
20:3 (*n*-6)	1.0±0.0	0.4±0.1	0.4±0.0	0.4±0.0	0.3±0.0	0.1±0.0	0.1±0.0	0.0±0.0
20:4 (*n*-6)	12.5±0.7	10.3±0.3	10.1±0.6	12.4±0.2	13.9±0.6	14.0±0.6	10.5±0.2	13.8±0.4
22:4 (*n*-6)	4.7±0.1	3.3±0.1	3.1±0.1	3.5±0.1	6.2±0.2	5.7±0.4	3.5±0.3	4.7±0.3
22:5 (*n*-6)	3.7±0.4	4.6±0.7	1.5±0.2	2.7±0.3	3.6±0.5	8.2±1.1	2.2±1.0	8.4±0.5
22:5 (*n*-3)	0.6±0.0	0.3±0.0	0.8±0.0	0.4±0.0	0.5±0.0	0.6±0.0	1.8±0.6	0.6±0.0
22:6 (*n*-3)	34.4±3.9	33.8±3.1	36.1±2.8	35.2±2.3	25.7±1.8	33.9±2.1	33.3±1.8	40.5±1.3
% Saturates	27.6±2.6	32.7±1.7	32.6±2.9	31.2±2.1	30.0±2.2	24.0±1.8	32.6±1.7	20.1±1.7
% MUFA	11.8±0.2	13.4±0.3	13.4±0.5	13.1±0.8	16.5±0.8	11.6±0.5	14.6±0.8	10.9±0.3
% PUFA	60.7±2.5	53.8±1.9	53.9±2.6	55.6±2.9	53.5±2.8	64.4±2.1	52.8±1.0	69.0±2.0
% *n*-9	6.3±0.1	6.8±0.1	7.4±0.3	8.2±0.5	9.8±0.4	7.7±0.6	9.8±0.7	6.5±0.1
% *n*-7	5.4±0.1	6.6±0.2	6.0±0.2	5.0±0.4	6.7±0.4	3.9±0.1	4.8±0.2	4.4±0.2
% *n*-6	25.7±1.4	19.7±1.2	17.0±0.9	20.0±0.6	27.4±2.0	29.9±2.0	17.4±2.1	27.9±1.9
% *n*-3	35.0±3.9	34.1±3.1	36.9±2.9	35.6±2.3	26.2±1.8	34.5±2.1	35.4±2.6	41.1±1.3
% Unsaturates	72.4±2.6	67.3±1.7	67.4±2.9	68.8±2.1	70.0±2.2	76.0±1.8	67.4±1.7	79.9±1.7
Unsaturation index	319±21	298±14	300±18	307±16	279±13	342±13	294±9	375±11
Chain length	19.7±0.1	19.5±0.1	19.5±0.1	19.6±0.1	19.4±0.1	20.0±0.1	19.5±0.1	20.2±0.1
20+22C PUFA	59.5±2.5	53.3±2.0	53.5±2.6	55.1±3.0	52.9±2.9	64.2±2.1	52.5±1.0	68.8±2.0
n-6/*n*-3	10.3±0.5	21.3±2.3	21.0±0.4	25.1±1.9	23.6±4.4	89.5±17	38.0±2.4	68.0±9.9
20:4/18:2	10.3±0.5	21.3±2.3	21.0±0.4	25.1±1.9	23.6±4.4	89.5±17	38.0±2.4	68.0±9.9

Values are means ± S.E.M., *N*=4 for all preparations except the zebra finch where *N*=3. Fatty acids are expressed as mol% of total fatty acids. Unsaturation index is the average number of double bonds per 100 fatty acid chains. Chain length is the average chain length of each fatty acid. Fatty acids where all species contained <0.5% are not included.

small endotherms compared to large endotherms, is associated with increased enzymic activity of the sodium pump in their respective tissues.

One of the goals of the current study was to determine if the higher Na^+,K^+-ATPase activity observed in small mammals and birds was the result of an increased concentration of sodium pumps, an increased molecular activity, or a combination of both. Compared with large mammals, small mammals had both a lower concentration of sodium pumps (Fig. 2) and an increased molecular activity in individual sodium pumps (Fig. 3), indicating that a combination of allometric changes in both these variables contributed to the body-size-related variation observed in Na^+,K^+-ATPase activity (Fig. 1). In birds there was a high degree of variation among the species and, as such, no significant relationships were found between body size and sodium pump density (Fig. 2) or molecular activity (Fig. 3). It is worth noting, however, that despite this variation, the allometric exponent measured for sodium pump density (–0.07) was equal to the exponent observed for Na^+,K^+-ATPase activity (Fig. 1), suggesting that changes in the concentration of sodium pumps may partly contribute to the allometric variation observed in brain Na^+,K^+-ATPase activity in birds.

The primary function of the sodium pump in the brain is the maintenance of the Na^+ gradient, which provides the immediate energy source for action potentials and supports the co-transport of various compounds such as amino acids (Clausen et al., 1991). Additionally the sodium pump also provides the gradient for Na^+/Ca^{2+} exchange in the brain (Clausen et al., 1991). In mammals the brain expresses a number of different isoforms (α_1, α_2 and α_3) that are thought to mediate the various processes performed by the sodium pump (Juhaszova and Blaustein, 1997). In intact mammalian brain, *in vivo* sodium pump activity has been estimated by isotope flux studies to be between 0.10–0.74 μmol ATP h^{-1} mg wet $mass^{-1}$ (Clausen et al., 1991). These values represent up to 50% of brain O_2 consumption, which is evidence of the importance of the sodium pump to brain function. Compared with the maximal *in vitro* activity values measured in the present study (Table 2), it can be estimated that *in vivo* the sodium pump is operating at less than 15% of maximum, although this may vary depending on which section of the brain is active at any particular time.

The allometric exponents for brain mass in the mammals and birds were 0.72 and 0.47, respectively. These allometric slopes are similar to previous investigations (Else and Hulbert, 1985;

Peters, 1983) and demonstrate that as body size increases, relative brain size decreases in both mammals and birds. Na^+,K^+-ATPase activity was combined with brain mass and expressed as the micromoles of inorganic phosphate liberated (from ATP) per brain per hour, and assuming that this value represented the maximum rate, and using a P/O ratio of 2.0 (Rolfe and Brown, 1997), it was possible to determine the potential maximal daily energy expenditure by the sodium pump (kcal day^{-1}). When these Na^+,K^+-ATPase values were compared with the basal metabolic rates from Table 1, it was found that potential maximal energy expenditure by the sodium pump represented approximately 25% of BMR in small species, but only approximately 8% of BMR in large mammals and birds. It should be noted however, that it is unknown whether all of the species are operating at a similar percentage of these maximal *in vitro* Na^+,K^+-ATPase activities, under *in vivo* basal conditions.

To examine if differences in sodium pump molecular activity were associated with alterations in membrane lipid composition, we also determined the fatty acid composition of microsomal membranes from the mammalian and avian brains. Phospholipid fatty acid composition has been shown to vary with body mass in a number of tissues (heart, skeletal muscle, kidney and liver) in mammals (Couture and Hulbert, 1995a; Hulbert et al., 2002b) and also in bird skeletal muscle (Hulbert et al., 2002a). Brain phospholipids from mammals however, show no body-size-related variation (Couture and Hulbert, 1995a; Hulbert et al., 2002b), and in the current study microsomal phospholipids from mammalian brains followed a similar pattern (Table 4), indicating that the lack of allometric variation is also manifest in subcellular membranes. The relationship between body size and membrane acyl composition has never previously been examined in avian brains, though the relative consistency of the fatty acid profile in the bird microsomes in the present study (Table 5), indicates that similar trends to those seen in mammals may also exist in birds.

The consistency of the fatty acid profile in the brain of the mammals and birds suggests that variations in membrane lipid composition are not associated with the significant allometric decline observed in molecular activity in the mammals or the inter-species differences observed for molecular activity in the birds in the current study. Indeed, in contrast with our previous work (Else and Wu, 1999; Wu et al., 2004), no significant correlations were observed between molecular activity values and fatty acid composition for the current data set. The reason for this lack of correlation is not clear, but is potentially related to other factors such as isoform differences between species.

Of interest was the consistency of the fatty acid profile in the brain of the mammals and birds. The exact mechanisms that maintain this constant membrane profile in the brain of different species are unknown at present. The composition of membranes is highly regulated and although the relative occurrence of various fatty acids may be influenced by their presence or absence in the diet, it is difficult to substantially alter phospholipid fatty acid composition through dietary manipulation. The main parameter that appears to be affected by diet is the relative percentage of *n*-6 and *n*-3 PUFA in the membrane (Hulbert et al., 2005), and interestingly the phospholipids from the sheep, cattle and geese all had higher levels of 22:5 (*n*-3) compared with the other species (Tables 4 and 5), which may indicate that these animals were pasture-fed, as forage crops are known to contain high levels of *n*-3 PUFA (Christie, 1981), and other tissues from these animals also contained high levels of long-chain *n*-3 PUFA (N.T., A.J.H. and P.L.E., unpublished).

Despite the relative consistency in fatty acid profile, there were a small number of significant allometric trends observed in the brains of birds, including a decrease in the content of 18:2 (*n*-6) and 20:3 (*n*-6), along with a significant increase in the ratio of 20:4 (*n*-6):18:2 (*n*-6) in the larger species. In mammals, the ratio of 20:4 (*n*-6):18:2 (*n*-6) gives an estimate of the activity of the Δ5 and Δ6 desaturase enzymes and the increased activity observed in the larger birds, coupled with the reduced levels of both 18:2 (*n*-6) and 20:3 (*n*-6) indicates a very active conversion of short-chain *n*-6 PUFA to their long-chain derivatives. This was very evident in the duck and emu where the highest ratio of 20:4 (*n*-6):18:2 (*n*-6) and also the highest levels of 22:5 (*n*-6) were seen (Table 5). Interestingly, while the estimated desaturase activity was increased in the larger birds, the elongase enzyme activity (estimated from the 18:0 : 16:0 ratio) was reduced in these species (results not shown). Whether elongase/desaturase enzyme systems operate in birds as they do in mammals is unknown, as within the mammals there was no allometric trend seen in the ratio of 20:4 (*n*-6) : 18:2 (*n*-6), while the ratio of 18:0:16:0 was actually increased in the larger mammals.

Comparison of the mammals and birds showed that the major differences were a greater content of 18:1 (*n*-9) in the mammals and higher levels of both 22:5 (*n*-6) and 22:6 (*n*-3) in the birds. Farkas et al. (2000) also found higher PUFA levels in birds compared to mammals and of particular interest in the current study was the high levels of 22:5 (*n*-6) observed in all of the birds (Table 5). Within the mammals the pig had the highest levels of 22:5 (*n*-6) along with the lowest levels of 22:6 (*n*-3) (Table 4). These fatty acids appear to be regulated in a reciprocal manner in the brain of mammals, where 22:6 (*n*-3) is the preferred long chain PUFA, but can be partially compensated for by 22:5 (*n*-6), if there is a dietary deficiency of *n*-3 PUFA (Carrié et al., 2000; Sheaff et al., 1995). While these fatty acids are similar in structure, recent work suggests that the additional double bond present in 22:6 (*n*-3), has a major impact on its physical properties, and as a result 22:5 (*n*-6) may not compensate functionally for 22:6 (*n*-3) with regards to lipid–protein interactions in the membrane (Eldho et al., 2003). The reason for the high levels of 22:5 (*n*-6) in the birds in the current study is unclear, however, it appears that the reciprocal relationship between 22:6 (*n*-3) and 22:5 (*n*-6) may not also be present in birds, as the highest levels of both fatty acids were found in the emu (Table 5).

Although there were a couple of small differences in fatty acid composition between the two vertebrate classes, it is clear

that there is a specific functional requirement for high concentrations of 22:6 (*n*-3) in the brain of both mammals and birds. Indeed 22:6 (*n*-3) appears to be prevalent in the brain of most vertebrates (Else and Wu, 1999; Farkas et al., 2000; Hulbert et al., 2002b). The high concentrations of this fatty acid in neural membranes is maintained both by astrocytes, which actively desaturate and elongate *n*-3 precursors and release the 22:6 (*n*-3) for uptake by the neurons (Moore, 1993), and by the neurons themselves, which preserve membrane 22:6 (*n*-3) in preference to other PUFA (Kim et al., 1999). Reductions in neural membrane 22:6 (*n*-3) have been linked with a number of functional deficits, both during developmental periods (Horrocks and Yeo, 1999) and during adulthood (Fenton et al., 1999; Hibbeln, 1998, 2002). While the exact membrane property related to 22:6 (*n*-3) that regulates neurological function is yet to be determined, it is potentially related to its influence on membrane proteins such as the sodium pump (Turner et al., 2003).

Another interesting finding in the current study was the high molar ratio of cholesterol:phospholipid observed in brain microsomal membranes from the mammals and particularly the birds (Table 3). These values were much higher than those seen in the kidney and heart of these animals (N.T., A.J.H. and P.L.E., unpublished), which is a trend that has been shown in other investigations (Wu et al., 2001; Yeagle, 1985). Cholesterol metabolism in the brain is quite complex, and recently it was suggested that cholesterol may be an essential factor in the formation and functioning of synapses (Pfrieger, 2003). Support for this hypothesis comes from studies which show that cholesterol turnover is much lower in the synaptic membranes of adults rats, compared with those of young rats, where presumably a large number of synapses are being established (Ando et al., 2002). Developing neurons synthesise their own cholesterol, however it is thought that neurons in adult brains derive their cholesterol from astrocytes (Pfrieger, 2002), which may in part explain the high levels of cholesterol observed in the present study, as astrocytes are the brain's most abundant cell type.

A potential functional explanation for the high level of cholesterol, is that it has been implicated as a major factor in the formation of lipid rafts. Lipid rafts represent membrane microdomains where saturated phospholipids, sphingomyelins and cholesterol aggregate and form less mobile, gel-like areas, with PUFA-rich phospholipids maintaining a very fluid environment in the remaining membrane (Simons and Ikonen, 1997). In the brain, lipid rafts are thought to be important in determining the appropriate distribution and orientation of post-synaptic receptors (Tsui-Pierchala et al., 2002). As mentioned above, brain phospholipids contain high levels of PUFA, particularly 22:6 (*n*-3) and to a lesser extent 20:4 (*n*-6), however cholesterol is relatively insoluble in these fatty acids (Brzustowicz et al., 2002a,b). Thus it is somewhat perplexing that in the brain there are high levels of both cholesterol and PUFA, but it has been suggested that this composition may facilitate sterol–lipid interactions in neural membranes, that promote lateral heterogeneity and the formation of functionally important lipid rafts (Huster et al., 1998). It is unknown at this stage whether lipid rafts influence the activity of the sodium pump, however, since both cholesterol and PUFA appear to be supplied to the neurons by astrocytes (Kim et al., 1999; Moore, 1993; Pfrieger, 2002), the exact importance of these glial cells in maintaining the appropriate neural membrane lipid composition requires further investigation.

Abbreviations

MUFA	monounsaturated fatty acids
PUFA	polyunsaturated fatty acids

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