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The causes and functions of mitochondrial proton leak

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Abstract

The non-linear relationship between respiration rate and protonmotive force in isolated mitochondria is explained entirely by Δp-dependent changes in the proton conductance of the mitochondrial inner membrane and is not caused by redox slip in the proton pumps. Mitochondrial proton leak occurs in intact cells and tissues: the futile cycle of proton pumping and proton leak accounts for 26% ± 7% of the total oxygen consumption rate or 33% ± 7% of the mitochondrial respiration rate of isolated hepatocytes (mean ± S.D. for 43 rats); 52% of the oxygen consumption rate of resting perfused muscle and up to 38% of the basal metabolic rate of a rat, suggesting that heat production may be an important function of the proton leak in homeotherms. Together with non-mitochondrial oxygen consumption, it lowers the effective P/O ratio in cells from maximum possible values of 2.33 (palmitate oxidation) or 2.58 (glucose oxidation) to as low as 1.1 in liver or 0.8 in muscle. The effective P/O ratio increases in response to ATP demand; the ability to allow rapid switching of flux from leak to ATP turnover may be an even more important function of the leak reaction than heat production. The mitochondrial proton conductance in isolated mitochondria and in hepatocytes is greatly modulated by thyroid hormones, by phylogeny and by body mass. Usually the reactions of ATP turnover change in parallel so that the coupling ratio is not greatly affected. Changes in proton leak in tissues are brought about in the short term by changes in mitochondrial protonmotive force and in the longer term by changes in the surface area and proton permeability of the mitochondrial inner membrane. Permeability changes are probably caused by changes in the fatty acid composition of the membrane phospholipids.

Key words: Mitochondrion; Proton leak; Redox slip; Metabolic rate, basal; Thyroid hormone; Phylogeny; Body mass

1. Introduction

In this paper we will discuss recent work from this laboratory to examine the nature and role of the leak of protons across the inner membrane of isolated mitochondria and of mitochondria in their natural intracellular habitat. We will be concerned with proton leak in liver and muscle, and not with the uncoupling protein, which is specific to brown adipose tissue and catalyses proton leak in a very different way. Our earlier work is reviewed in Refs. [1–3].

Oxidative phosphorylation is the synthesis of ATP from ADP and P_i by mitochondria (or bacteria) driven by electron flow from a reduced substrate to oxygen. Electron transport is coupled to pumping of protons from the mitochondrial matrix, forming an electrochemical potential difference for protons across the mitochondrial inner membrane, known as the protonmotive force, Δp. Protonmotive force drives the protons back into the matrix through the ATP synthase and the combined action of the adenine nucleotide and phosphate carriers, resulting in the formation of extramitochondrial ATP. If the electron transport reactions always led to proton pumping, and if all proton backflow was always through these phosphorylation reactions, then the processes of substrate oxidation and ADP phosphorylation would be completely coupled. However, it is clear that oxidative phosphorylation is never fully coupled either in isolated mitochondria or in mitochondria in their natural intracellular environment.

2. Proton leak and redox slip

The simplest illustration of this imperfect coupling is the observation that isolated mitochondria still con-

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tinue to consume oxygen at a low rate even when they are prevented from synthesising ATP by lack of ADP (state 4) or by addition of oligomycin (an inhibitor of proton flow through the membrane domain of the ATP synthase). In these non-phosphorylating steady states, electron transport might be occurring without the associated proton pumping; this is known as a slip reaction in the proton pumps. Alternatively, the protons might still be pumped at a low rate, but would then flow back into the matrix by pathways not involving ATP synthesis; this is known as proton leak.

The properties of this imperfect coupling can be analysed using an experimental approach first reported by Nicholls [4]. The substrate oxidation reactions of mitochondria in state 4 can be progressively slowed by addition of electron transport inhibitors such as malonate (which inhibits succinate dehydrogenase), myxothiazol (which inhibits the cytochrome bc_1 complex) or cyanide (which inhibits cytochrome oxidase). As the rate of substrate oxidation decreases, the rate of proton pumping decreases and so the value of Δp falls. Analysis of the relationship between substrate oxidation rate and Δp shows that it is non-linear: at higher values of Δp (and higher rates of electron transport) the oxygen consumption rate is much greater (or the value of Δp is much less) than expected if there was a linear dependence. The fact that respiration rate in state 4 is finite but sub-maximal tells us that at least part of the imperfect coupling is due to proton leak reactions. This is because state 4 respiration rate in the complete absence of proton leak would be either zero (less than complete slip in the redox proton pumps) or maximal (complete slip in the pumps). The non-linearity between substrate oxidation rate and Δp can be explained in four different ways [5]: (i) increased proton conductance at higher Δp [1–5]; (ii) the appearance of redox slip reactions at higher Δp [6,7]; (iii) increased proton conductance at higher electron transport rates [8,9] or (iv) the appearance of slip reactions at higher electron transport rates [10]. Up to now, these possibilities have been hard to test because of experimental difficulties in measuring proton leak rates directly.

We have introduced a simple titration method that can distinguish between these different possibilities [5,11]. The method looks at the respiration needed to drive proton flow at different values of Δp through an added proton ionophore (carbonylcyanide *m*-chlorophenylhydrazone, CCCP) that is known to have a linear dependence of proton flow on Δp. It turns out that redox slip in the mitochondrial proton pumps at higher Δp would cause the CCCP-dependent respiration to be non-linearly related to Δp whereas proton leak would cause it to be linearly related. Rate-dependent changes in slip or leak would give lines with different slopes depending on the CCCP concentration. The results [5,11] (Fig. 1) show that in isolated mitochondria only the first of the four explanations is correct: all of the non-linearity in the state 4 titration curves of respiration rate against Δp is caused by

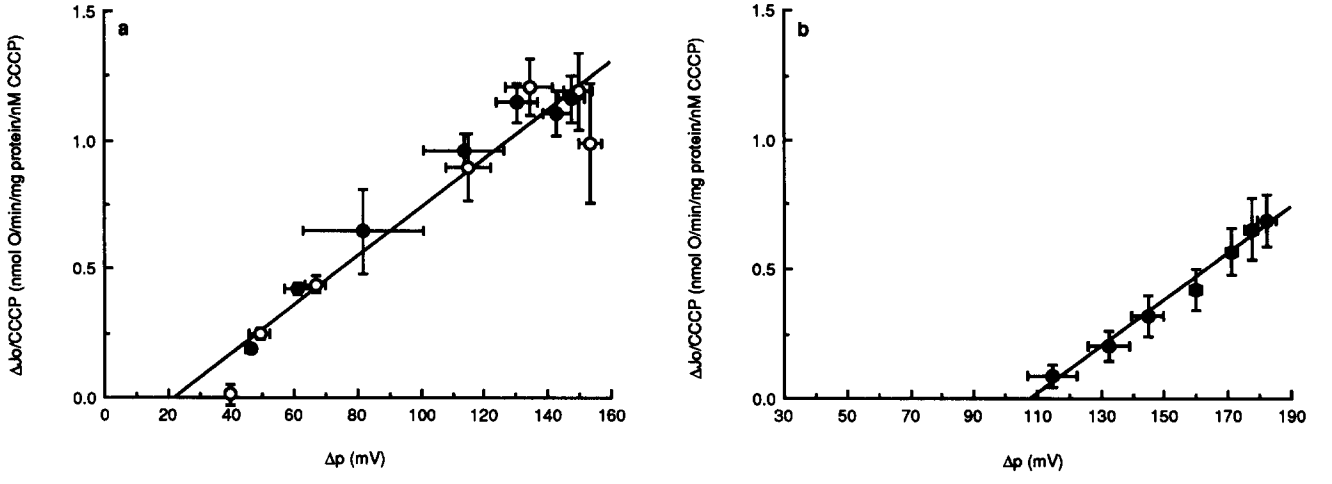

Fig. 1. Test for redox slip in rat liver mitochondria respiring on succinate with Δp measured with (a) ^{86}Rb in the presence of valinomycin or (b) TPMP using an electrode. The extra respiration rate needed to drive proton flow through added CCCP divided by CCCP concentration (ΔJ_o/CCCP) is plotted against Δp. This plot gives lines with slopes inversely proportional to the H^+/O ratio (see [5]). In (a) mitochondria were titrated with malonate at 37°C as described in [5]. ΔJ_o/CCCP and Δp were measured in the presence of 20 nM (○) or 40 nM (●) CCCP. Membrane potential was measured from the distribution of ^{86}Rb in the presence of valinomycin and ΔpH was calculated from a calibration curve [5]. Points represent mean and S.E. of three independent experiments. The slope of the line was 9.5 pmol O/min per mg per nM CCCP per mV. In (b) mitochondria were incubated at 2 mg protein/ml at 37°C in medium containing 125 mM KCl, 3 mM Hepes, 5 mM KP_i, 1 mM EGTA (pH 7.4) together with 5 μM rotenone, 500 ng oligomycin/mg protein, 50 ng nigericin/mg protein, 4 mM succinate and 5 μM TPMP. Membrane potential was measured with an electrode sensitive to TPMP and ΔpH was assumed to be zero. ΔJ_o/CCCP and Δp were titrated with malonate in the presence of 25 nM CCCP. Points represent mean and S.E. of three independent experiments. The slope of the line was 9.1 pmol O/min per mg per nM CCCP per mV.

Δp-dependent changes in the passive mitochondrial proton conductance and none is due to redox slip or rate-dependent effects.

Fig. 1 reports the results of experiments to test for any redox slip induced by Δp or respiration rate in isolated rat liver mitochondria respiring on succinate. We have plotted the extra respiration induced by CCCP divided by CCCP concentration (ΔJ_o/CCCP) against Δp. This plot gives lines with slopes inversely proportional to the H^+/O ratio (see Refs. [5,11]). Fig. 1 shows the behaviour of mitochondria in experiments in which Δp was measured either from the distribution of Rb in the presence of valinomycin measured using radiolabels (Fig. 1a) or from the distribution of the lipophilic probe methyltriphenylphosphonium (TPMP) measured with an ion-sensitive electrode (Fig. 1b). In both cases the lines are straight and thus the H^+/O ratio is invariant as Δp changes, showing that there is no Δp-dependent redox slip in the proton pumps. In addition, the lines in Fig. 1a for different CCCP concentrations superimpose, showing that neither endogenous proton leak nor the H^+/O ratio vary with the rate of substrate oxidation. The fact that the slopes of the lines in Fig. 1a and Fig. 1b are the same strongly suggests that the intercept in Fig. 1b is an artefact of the electrode methodology and can be ignored. Thus the results in Fig. 1 confirm our earlier conclusion [5,11] that there is no redox slip in the mitochondrial electron transport chain under any of the conditions that we have examined, and the non-linear titrations of state 4 respiration rate and Δp are completely explained by the Δp-dependence of the mitochondrial proton leak.

3. The contribution of mitochondrial proton leak to the respiration rate of cells

The proton leak reaction is present in mitochondria isolated from different organs and species [12–17]. One pertinent question that is levelled at mitochondrial studies of this kind is whether the proton leak is an artefact of isolated mitochondria (hence the relentless quest for improved isolation techniques and incubation conditions to achieve high respiratory control ratios) or whether proton leak is a real physiological phenomenon. Is it caused by damage during mitochondrial isolation, or is it a property of mitochondria within cells? We have shown that the properties of the mitochondrial proton leak in intact hepatocytes and thymocytes are much the same as they are in isolated liver mitochondria, clearly showing that the leak is not an isolation artefact [18–24].

But how important is this mitochondrial proton leak reaction in intact cells? To answer this question we have measured how much of the oxygen consumption of hepatocytes is used to drive the proton leak, and the quantitative control exerted by the leak over respiration rate [19–24]. A surprisingly high proportion of oxygen consumption is used to drive the leak in resting hepatocytes, between 20% and 40%. The mean proportion of total oxygen consumption rate used to drive mitochondrial proton leak in resting hepatocytes from 43 normal rats was 26.1% ± 6.5% (S.D.) and the mean proportion of mitochondrial respiration rate after subtraction of non-mitochondrial oxygen consumption used to drive proton leak was even higher: 33.3% + 6.8% (data from [19,21,23] and Porter and Brand, unpublished observations). The leak has significant control over respiration rate in normal resting hepatocytes, with a flux control coefficient of 0.24 ± 0.05 (S.D.) (data from 32 rats [21,23]), showing that changes in the leak kinetics could cause significant changes in the oxygen consumption rate of the cells; this value is similar to that in isolated liver mitochondria at respiration rates intermediate between state 4 and the active state 3 [25]. The non-mitochondrial oxygen consumption rate in resting hepatocytes is 20.9% ±3.3% (S.D.) of the total oxygen consumption rate (data from 37 rats [19,21,23]).

Since oxygen consumption that is used to drive the futile cycle of proton pumping and leak across the mitochondrial membrane cannot be used to drive ATP synthesis, the occurrence of the proton leak in intact cells lowers the effective P/O ratio below its maximum possible values. If 4 protons must enter the matrix to form each extramitochondrial ATP, and if 4 protons per O consumed are pumped by Complex I and 6 are pumped by the rest of the chain, then the maximum possible value for the P/O ratio in hepatocytes oxidising palmitate would be 2.33 [26]. The maximum value for glucose oxidation would be 2.58 [22]. Proton leak and non-mitochondrial oxygen consumption together decrease these values by 47% ± 7% (data above), so that the effective P/O ratio in hepatocytes is only about 1.1 to 1.4 [22,26]; much lower than the value of 3.0 that is often assumed.

Increased ATP demand will decrease the value of Δp and so decrease the proton leak rate. This raises the effective P/O ratio, showing how oxidative phosphorylation varies its coupling efficiency depending on demand without needing to make very large changes in oxygen consumption rate. On the other hand, increased proton leakiness will divert more of the energy flux to futile proton cycling, so lowering the effective P/O ratio. The very rapid, short-term variation in coupling efficiency on demand without great changes in rate of oxygen consumption, and the longer-term variation in the coupling efficiency by modulation of proton leakiness may be important functions of the proton leak in cells. This is quantitatively reflected by control coefficients: the effective P/O ratio in hepato-

cytes is controlled both by ATP demand (control coefficient 0.31) and by proton leak (control coefficient −0.34) but hardly at all by the reactions of substrate oxidation (control coefficient 0.03) [22].

4. The contribution of mitochondrial proton leak to the basal metabolic rate of animals

About 40% of the respiration rate of a rat is used to provide the ATP needed to drive protein synthesis [27] and the plasma membrane sodium and calcium ATPases [28]. It is less clear what other processes are being driven by the energy released during substrate oxidation. The liver is an important contributor to the animal's oxygen consumption, accounting for about 15% of the respiration rate [29]. If the proportion of liver respiration used to drive mitochondrial proton leak is similar to the proportion doing so in isolated resting hepatocytes (26%), then mitochondrial proton leak in liver alone accounts for about 4% of basal metabolic rate in the rat.

The major oxygen-consuming organ in the rat is skeletal muscle, accounting for about 45% of resting respiration [29]. Is mitochondrial proton leak a significant contributor to muscle oxygen consumption? The properties of proton leak in isolated skeletal muscle mitochondria are quite similar to those in liver and other tissues [13], suggesting that muscle mitochondrial proton leak might be a significant contributor to basal metabolic rate in the rat. We have recently attempted to assay mitochondrial proton leak in an intact perfused muscle system to see if this is so (Rolfe and Brand, unpublished observations).

Fig. 2 shows the result of the experiment. Rat hindquarter perfused with TPMP (a probe for intracellular mitochondrial membrane potential) in a recirculating system reaches a steady state for oxygen consumption rate in about 10 min and for TPMP uptake in about 60 min. If we then add sufficient oligomycin to prevent mitochondrial ATP synthesis, respiration rate decreases by an average of about 15% and TPMP accumulation increases, reflecting an increased intracellular mitochondrial membrane potential. To determine how much of the original rate of oxygen consumption was being used for ATP synthesis, we titrate out the TPMP signal to its original value using cyanide (an inhibitor of electron transport), and measure the new respiration rate at the original membrane potential but in the absence of oxidative ATP synthesis. Fig. 2 shows that only about 34% of the oxygen consumption rate of the perfused resting muscle is used for ATP synthesis. The remaining 66% must be used for other purposes. About 14% of the respiration rate is insensitive to cyanide, and is attributed to non-mitochondrial reactions. The rest, 52% of the total, represents the respiration rate of this muscle preparation that is needed to drive the futile cycle of proton pumping and leak across the mitochondrial inner membrane within the intact tissue. The system is complex, and the assumptions involved in quantifying the leak rate are difficult to test unambiguously, but it is clear

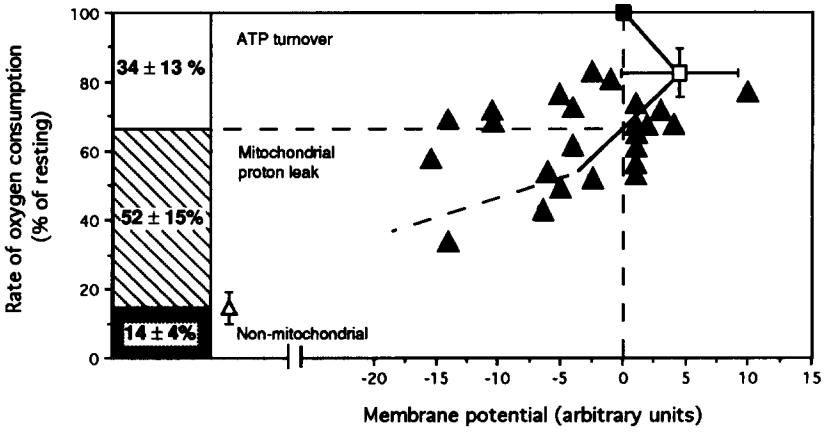

Fig. 2. Measurement of the contributions of ATP turnover, mitochondrial proton leak and non-mitochondrial oxygen consumption to the resting oxygen consumption rate of perfused rat skeletal muscle. The hindquarter of an adult rat was perfused, using a recirculating system, with Krebs-Henseleit bicarbonate buffer at 37°C containing 10 mM glucose, 10 mM lactate, 1 mM pyruvate, 40 mg defatted bovine serum albumin/ml and 1 μM TPMP (pH 7.2), gassed with 95% O_2/5% CO_2 (v/v). Oxygen consumption rate was measured in the perfusate with an oxygen electrode and perfusate TPMP concentration (an indicator of intracellular mitochondrial membrane potential) was measured using a TPMP-sensitive electrode. For each experiment, all data are normalised to the resting oxygen consumption rate and membrane potential (■). ATP production by oxidative phosphorylation was then inhibited by adding excess oligomycin (about 25 μg/g tissue) (□). Membrane potential was manipulated to bring it back to the resting value by progressively inhibiting the mitochondrial respiratory chain with sodium cyanide (▲). The non-mitochondrial oxygen consumption rate was determined in the presence of excess (1–2 mM) sodium cyanide (△). Points with excess oligomycin (□) and cyanide (△) are means (±S.E.) of seven and three independent experiments, respectively. All other data are shown as individual points. Membrane potential was not determined when measuring non-mitochondrial oxygen consumption.

that a large proportion of the oxygen consumption of resting rat skeletal muscle is used to drive mitochondrial proton leak.

Analysis of the full dataset shows that the mitochondrial proton leak in resting muscle has quite strong control over oxygen consumption rate (0.26) and over the value of the effective P/O ratio (−0.72). The effective P/O ratio is only 34% (using total oxygen consumption) or 40% (using mitochondrial oxygen consumption) of the maximum possible value, and so has a value of about 0.8 to 1.1, very much lower than is widely assumed. Thus, the role of mitochondrial proton leak in energy metabolism is even more prominent in intact muscle than it is in liver.

Together, liver and muscle account for about 60% of the resting oxygen consumption of a rat [29]. Our results suggest that mitochondrial proton leak in these two tissues accounts for 27% of the basal metabolic rate of the animal. If the proportion of oxygen consumption used to drive proton leak in the other tissues is similar to the proportion in liver, then as much as 38% of BMR may be caused by mitochondrial proton leak in different tissues. This suggests another prominent function of mitochondrial proton leak, as the single most important futile cycle driving respiration rate and the production of metabolic heat in homeotherms [2].

5. Factors affecting mitochondrial proton leak rate under different conditions

There is a hierarchy of different timescales over which the rate of proton flow through the mitochondrial proton leak pathways can be modified. These range from immediate responses of leak rate to changes in Δp caused indirectly by changes in rates of other reactions in the system, through direct effects of artificial and natural compounds such as uncouplers on membrane leakiness, to changes in leakiness occurring over a few days in response to hormones, right up to permanent differences in leak kinetics reflecting evolutionary history.

Immediate changes in leak rate occur if Δp is perturbed even in the absence of any change in the kinetics of the proton leak itself. The reactions that produce and consume Δp are all very responsive to its value and the steady state that is maintained represents a very fine balance of fluxes. Any kinetic change in one block of reactions caused by changes in substrate supply or ATP demand will cause a small change in Δp, and this in turn will change the rate of the other blocks of reactions. As discussed above, this is why the effective P/O ratio (or coupling ratio) is so sensitive to ATP demand: increased ATP demand causes an increase in the rate of the phosphorylation reactions at any Δp, so lowering Δp and proton leak and increasing the coupling ratio. The overall kinetic responses to Δp of the proton leak reactions and the reactions that synthesise and consume ATP seem well-matched in cells, so that any increase in substrate supply alters them both to the same extent with little effect on the coupling ratio.

Direct effects of natural uncouplers would increase the rate of the proton leak at a given value of Δp, and so would alter the balance of energy distribution between ATP synthesis and proton leak. In this way it would be possible to adjust the set point for the coupling ratio, for example to alter the rate of heat production at constant ATP output or the rate of ATP output at constant heat production. Similar effects could be achieved by altering the kinetics of the other reaction blocks. The uncoupling protein of brown adipose tissue represents an example of a well-studied natural uncoupler; in other tissues different natural uncoupling mechanisms may operate.

Some hormones cause changes in membrane leakiness over a few days. The best studied example is thyroid hormone treatment of rats [24,30], which causes changes in mitochondrial proton permeability in isolated rat liver mitochondria [31,32] and in hepatocytes [19,22–24]. The decrease in mitochondrial proton leakiness in hepatocytes from hypothyroid rats accounts for the whole of the change in mitochondrial oxygen consumption in these cells [23]; it changes the balance between ATP production and heat production, so raising the effective P/O ratio by 10 to 15% [22] without any changes in the underlying maximum stoichiometric efficiency of energy coupling [33]. In cells from hyperthyroid animals, however, the increased respiration rate is caused by matched alterations in proton leakiness and in ATP turnover kinetics, so that increased rates of ATP turnover occur without significant alterations in the coupling ratio [23,34]. This is an example of an unchanged set point for the coupling ratio, even though the rate of oxygen consumption has almost doubled.

Permanent or semi-permanent differences in mitochondrial proton permeability are seen in animals of different species that operate at different basal metabolic rates. We have identified two separate examples, one due to phylogeny [17] and the other due to differences in body mass [15].

Reptiles have basal metabolic rates that are in general 4- or 5-times lower than those of mammals of the same body mass, even if consideration is restricted to reptile species that have a preferred body temperature of 37°C. One such reptile is the bearded dragon, a lizard of central Australia, which is comparable in body mass and body temperature to the laboratory rat but has a 7-fold lower basal metabolic rate. Dragons have mitochondria with a smaller ratio of inner membrane

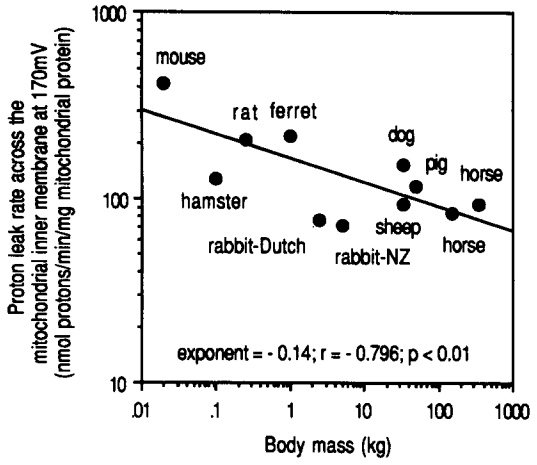

Fig. 3. Relationship between body mass of animals of different species and the rate of proton leak in isolated liver mitochondria at a potential of 170 mV. For details see Ref. [15]. The line was fitted to weighted data by linear regression.

surface area to matrix volume, less mitochondria per gram of tissue and less of the metabolically active tissues per animal, explaining in part their lower metabolic rates [35]. We have shown that dragon liver mitochondria are 4- to 5-times less proton-permeable than rat liver mitochondria [17]. Dragon hepatocytes respire 4-fold more slowly than rat hepatocytes, but seem to devote a similar proportion (less than 30%) of their respiration rate to the futile proton cycle across the mitochondrial membrane. This suggests that the activity of the ATP turnover reactions in dragon cells is lower by the same factor so that the set point for the coupling ratio is not disturbed too much. If this is so, then heat production is not the most important reason for submaximal coupling ratios in mammals, since dragons have no need of metabolic heat production to maintain their body temperature but have a similar effective P/O ratio.

Larger mammals have lower mass-specific basal metabolic rates than smaller ones; a horse with a mass of 200 kg has a 10-fold lower oxygen consumption per gram than a mouse with a mass of 20 g. This is paralleled by a lower mitochondrial content in larger animals [36]. We have shown that the lower metabolic rates are reflected in the proton permeability of mitochondria from animals of different mass; liver mitochondria from mice are about 4-fold more leaky to protons than liver mitochondria from horses [15]. The full dataset for all the animals we have examined is shown in Fig. 3. To establish whether these differences in mitochondrial proton leakiness were present in intact tissues, we prepared hepatocytes from different species and measured the oxygen consumption used to drive the proton leak cycle within the cells (Porter and Brand, unpublished observations). Fig. 4a shows that there is a very clear difference in the oxygen consumption rate devoted to mitochondrial proton leak, mouse hepatocytes consume oxygen 10-times faster for this purpose than do horse hepatocytes. Fig. 4b shows the proportion of cellular oxygen consumption that is used for proton leak in the different species. There is no trend for the proportion to change with body mass, instead the proton leak cycle accounts for about 15 to 30% of cellular oxygen consumption in all cases. Thus, to change basal metabolic rate, evolution has changed both ATP-consuming reactions and mitochondrial proton leak in parallel. It seems that the set point for the coupling ratio is maintained despite large changes in

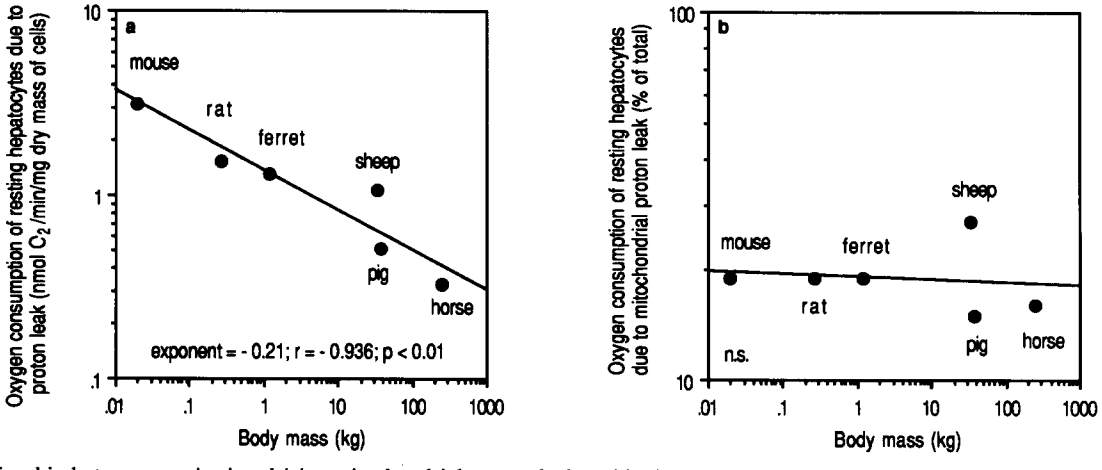

Fig. 4. Relationship between respiration driving mitochondrial proton leak and body mass in resting hepatocytes isolated from different species. Hepatocytes were isolated essentially according to [37]. Incubation conditions and measurement of the contribution of mitochondrial proton leak to resting oxygen consumption were as in [23]. In (a) the absolute values of oxygen consumption needed to drive proton leak at resting mitochondrial membrane potential are plotted as a function of body mass. In (b) the proportion of the total resting oxygen consumption due to proton leak is plotted as a function of body mass. Lines were fitted by linear regression.

the cellular respiration rate, emphasising the importance of the mitochondrial proton leak as an important cellular function.

6. Mechanisms of changes in mitochondrial proton leak rate

To change mitochondrial proton leak rate in a cell or an animal you can change the total area of mitochondrial inner membrane across which leak occurs, the value of Δp (the driving force for the leak) or the permeability of the membrane itself. Changes in total inner membrane area in cells and animals of different species and changes in the value of Δp certainly occur; they are discussed above. Changes in the apparent permeability of isolated mitochondria (expressed conventionally per mg mitochondrial protein) can be caused by changes in the area of mitochondrial inner membrane per mg protein. Such changes account for less than half of the effects of thyroid hormone treatment [38], two-thirds of the effects of body mass [39], and none of the differences between mitochondria isolated from rats and dragons [17]. In each case, changes in the permeability of the membrane itself appear to be responsible for the remainder of the observed changes in proton permeability in isolated mitochondria. Neither the mechanism of the proton leak itself [3] nor the mechanism of the change in proton leakiness is fully understood, but there is a striking correlation between membrane proton permeability and the fatty acid composition of the membrane phospholipids [17,38–41] suggesting that changes in phospholipid/phospholipid or phospholipid/protein packing may be responsible. This possibility is currently under test.

7. Conclusions

There is no good evidence for redox slip reactions in oxidative phosphorylation; Δp-dependent proton leak reactions appear to be the only important cause of imperfect coupling under any of the conditions we have examined. The mitochondrial proton leak is not an artefact of isolation, but occurs in intact liver cells and muscles. It induces a futile cycle of respiration-driven proton pumping and proton leak that makes a substantial contribution to cellular respiration. Indeed, it seems that it may be the single most important consumer of free energy supply in animals. In all the situations in which proton leak changes there is a pattern. The proportion of cellular respiration that is devoted to mitochondrial proton leak sets the effective P/O ratio, and this set coupling ratio is protected despite huge changes in the absolute rates of oxygen consumption. However, in the short term the coupling ratio is a variable, changing in response to the second-by-second needs of the cell for ATP; when ATP demand increases the coupling ratio rises and when ATP demand decreases the coupling ratio falls. Removal of thyroid hormones alters the set point, allowing the cell to divert more of its energy flow into ATP synthesis; the cell can also alter the set point by using natural uncouplers such as the uncoupling protein of brown adipose tissue. Matching of the proton leak to the anticipated rates of ATP turnover to keep the resting coupling ratio constant despite large differences in respiration rates is achieved by variation in the surface area of mitochondrial inner membrane in the cell and by variation in the proton permeability of the membrane. Changes in proton permeability may be caused by changes in the composition of the fatty acids of the membrane phospholipids.

Acknowledgements

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