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Changes in the Hepatic Mitochondrial Respiratory System in the Transition from Weaning to Adulthood in Rats

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In the present study we investigated the changes in the hepatic mitochondrial respiratory system in the transition from weaning to adulthood in the rat. We conceptually divided the system into blocks of reactions that produced or consumed mitochondrial membrane potential and then measured the kinetic responses of these blocks of reactions to changes in this potential in isolated liver mitochondria from 25- and 60-day-old rats using succinate as substrate. Moreover, we considered the mitochondrial membrane potential producers to be divided into blocks of reactions that reduced or oxidized ubiquinone-2 (Q-2) and then measured the kinetic responses of these two blocks to changes in Q-2 redox state as well as the flux control coefficients and the cytochrome content. We found that adult rats exhibited significantly higher state 3 respiratory rates with increased kinetic response of the substrate oxidation pathway to the mitochondrial membrane potential, slightly decreased activity of the phosphorylating system, increased kinetic responses of both Q-2 reducers and oxidizers to Q-2 redox state, and increased cytochrome content. Our results indicate that important changes in the hepatic mitochondrial respiratory system occur in the transition from weaning to adulthood in rats.

Key Words: **mitochondria; oxygen consumption; top-down elasticity analysis; weaning–adulthood transition.**

During development and aging in mammals, body energy requirements undergo modifications in response to changes in physiological functions (1). Since liver has important roles in intermediary energy metabolism and mitochondria are the major sites of metabolic energy production, several studies have been carried out in order to assess the morphological and functional changes in the liver mitochondrial compartment during development and aging. In particular, most of these studies have been centered on the first hours after birth (2), the suckling–weaning transition (2), and aging (3). The following modifications of the hepatic mitochondrial compartment have been shown to occur immediately after birth: decreased mitochondrial volume, increased number of mitochondria per hepatocyte (4–6), increased state 3 respiration (7), and decreased passive proton permeability of mitochondrial membranes (8). Moreover, the suckling–weaning transition is essentially coupled with a decreased mitochondrial fatty acid oxidation (9, 10) and ketone body production (11). In addition, liver mitochondria isolated from old mammals essentially show a fall in the respiratory capacity (12, 13) and in the maximal rate of ATP synthesis (13).

In contrast to the number of studies carried out on these specific developing and aging periods, to our knowledge, relatively little work that fully investigates developmental changes in the hepatic mitochondrial compartment in the transition from weaning to adulthood in rats has been performed. Studies of this nature should also help in determining if the above developmental period is coupled with changes in liver activity. In light of the above considerations, we thought it would be interesting to study functional variations in the liver mitochondrial compartment in 25- and 60-day-old rats.

To this purpose, we measured respiration rates and analyzed the hepatic mitochondrial respiratory system by using the top-down elasticity approach [for review see (14)] in isolated mitochondria using succinate as substrate. Top-down elasticity analysis allows the identification of the sites of action of external effectors within metabolic pathways and has been previously

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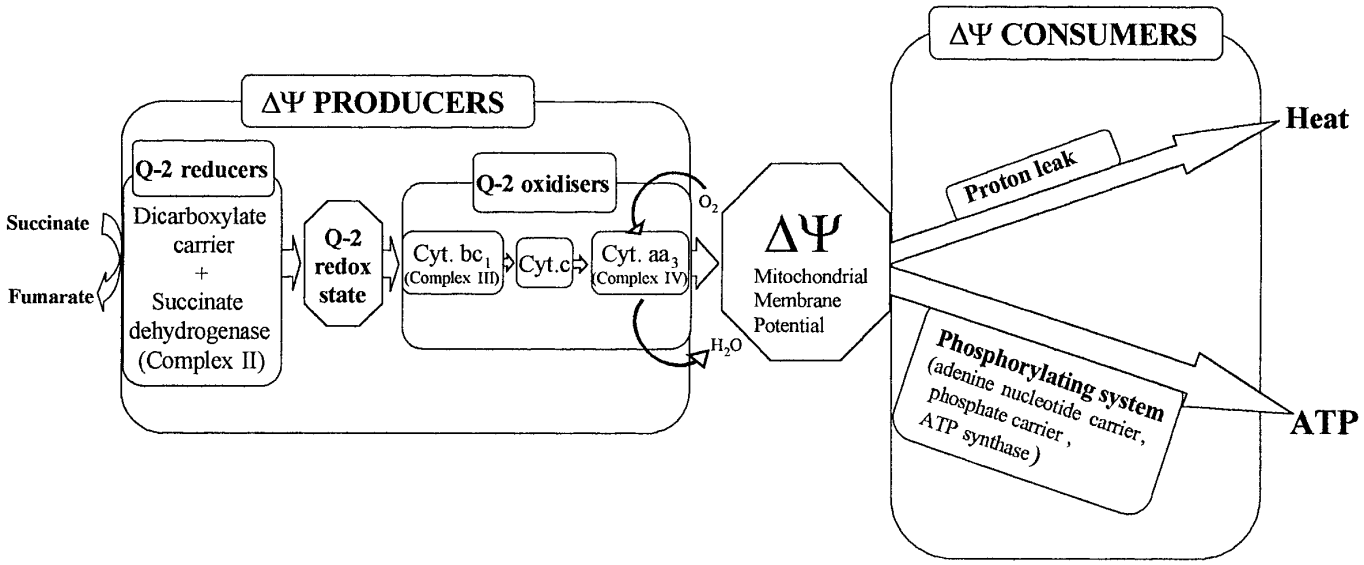

FIG. 1. The division of the mitochondrial respiratory system into blocks of reactions. The mitochondrial respiratory system can be divided into blocks of reactions which produce and consume the common intermediate, the mitochondrial membrane potential ($\Delta\Psi$). The $\Delta\Psi$ is produced by the substrate oxidation pathway, which consists of all of the steps from added succinate to $\Delta\Psi$. Then the $\Delta\Psi$ is consumed by the phosphorylating system and the proton leak. The blocks of $\Delta\Psi$ producers can be further divided into two blocks of reactions which reduce and oxidize the common intermediate Q-pool (expressed as Q-2 redox state).

used to investigate the mechanism by which thyroid hormones (15–18), glucagon (19), and high-fat diet (20) alter the respiration rates of isolated liver mitochondria.

In this paper, we applied top-down elasticity analysis by conceptually dividing the mitochondrial respiratory system into two groups of reactions (Fig. 1): (1) the reactions that produce the intermediate "mitochondrial membrane potential" or $\Delta\Psi$ (i.e., the substrate oxidation pathway), called $\Delta\Psi$ producers, and (2) the reactions that consume the intermediate $\Delta\Psi$ either producing ATP (the phosphorylating system) or not producing ATP (the proton leak), called $\Delta\Psi$ consumers. Hence, we measured the kinetic responses of these blocks of reactions to changes in $\Delta\Psi$ in mitochondria isolated from 25- and 60-day-old rats. We then extended the top-down approach to analyze in more detail the $\Delta\Psi$ producers. To this end, we conceptually divided the substrate oxidation pathway (from succinate to oxygen) in uncoupled mitochondria into two blocks of reactions connected by the common intermediate, Q-2[2] redox state: (1) the block of reactions which reduce the Q-2, called Q-2 reducers, and (2) the block of reactions which oxidize it, called Q-2 oxidizers (Fig. 1). The kinetic responses of these two blocks of reactions to changes in the common intermediate Q-2 were then determined. In addition, we measured the flux control coefficients of Q-2 reducers and Q-2 oxidizers and the cytochrome content in both sets of mitochondria.

[2] Abbreviations used: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; TPMP,$^+$ triphenylmethylphosphonium cation; Q-2, ubiquinone-2.

MATERIALS AND METHODS

Animals. Male Wistar rats (Harlan, Cambridge, UK) were used for the experiments. They were housed at 24°C under an artificial circadian 12-h light–12-h dark cycle, with *ad libitum* access to water and a standard stock diet. Rats of 25 or of 60 days of age were killed by cervical dislocation. The order in which 25- and 60-day-old rats were killed was random.

Preparation of mitochondria. Mitochondria were prepared simultaneously from 25- and 60-day-old rats. Livers were quickly removed, weighed, finely minced, and washed with a medium containing 220 mM mannitol, 70 mM sucrose, 20 mM Hepes, pH 7.4, 1 mM EDTA, and 0.1% fatty acid-free bovine serum albumin. Tissue fragments were gently homogenized with the same medium (1/10, w/v) in a large Dounce homogenizer with medium-fit plunger. The homogenate was filtered through sterile gauze and freed of debris and nuclei by centrifugation at $1000g_{av}$ for 10 min; the resulting supernatant was centrifuged at $3000g_{av}$ for 10 min. The mitochondrial pellet was washed twice and finally resuspended in a medium containing 80 mM KCl, 50 mM Hepes, pH 7.0, 5 mM KH_2PO_4, 1 mM EGTA. The protein content of the mitochondrial suspension was determined by the method of Hartree (21) using bovine serum albumin as the protein standard.

Measurement of oxygen consumption and mitochondrial membrane potential. Mitochondrial oxygen consumption was measured polarographically with a Clark-type electrode (Rank Brothers, Bottisham, Cambridge). $\Delta\Psi$ was measured with an electrode sensitive to methyltriphenylphosphonium cation ($TPMP^+$) as described by Brown and Brand (22). This was coupled to a reference electrode, and both electrodes were inserted through an airtight port into the oxygen vessel to monitor oxygen consumption and $\Delta\Psi$ simultaneously.

Incubations were carried out at 30°C, with addition of 0.5 mg/ml

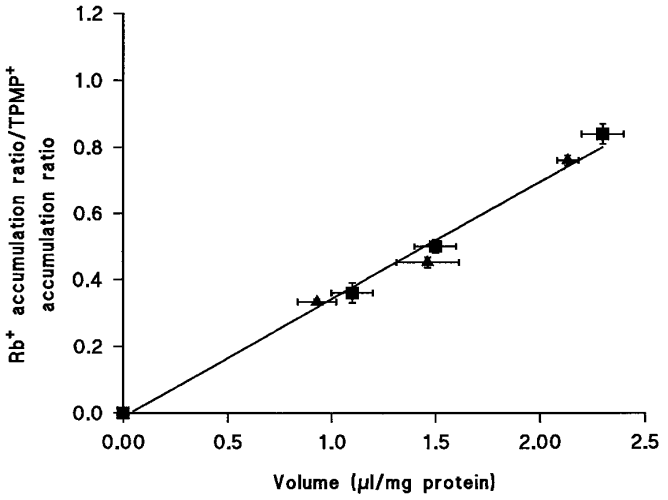

FIG. 2. Ratio of Rb^+ to $TPMP^+$ accumulation as a function of matrix volume in liver mitochondria isolated from 25-day old (▲) and 60-day-old (■) rats. Mitochondria (1 mg of protein/ml) were incubated for 2 min in medium containing various concentrations of sucrose (100–300 mM), 5 μM rotenone, 10 mM succinate, 5 μM TPMP, 100 pmol/mg valinomycin, 0.1 μCi/ml [H^3]TPMP, 0.04 μCi/ml ^{86}Rb. Matrix volume was determined in parallel incubation with 1 μCi/ml ^3H_2O and 0.1 μCi/ml [^{14}C]sucrose replacing [H^3]TPMP and ^{86}Rb. Reactions were stopped by rapid centrifugation for 2 min, and the supernatant and the pellet were assayed for radioactivity in a scintillation counter (24). Each point is the mean ± SE of three different experiments, each carried out in six replicates. The slope of the line [0.35 $(\mu l/mg)^{-1}$] is the TPMP-binding correction and is the same for both sets of mitochondria.

mitochondrial protein to 3.5 ml of the above suspension medium supplemented with 0.1% defatted bovine serum albumin. Stock 9% bovine serum albumin was defatted by the method of Chen (23). The incubation medium also contained 5 μM rotenone and nigericin (80 ng/ml), which was used to collapse the pH difference across the mitochondrial inner membrane and allow the whole of the proton motive force across the mitochondrial inner membrane to be expressed as $\Delta\Psi$.

Uptake of $TPMP^+$ was corrected for binding by using a factor of 0.35 for both sets of mitochondria determined from the ratio of Rb^+ to $TPMP^+$ accumulation as a function of mitochondrial volume (Fig. 2), as described by Brown and Brand (24). $\Delta\Psi$ was then calculated as $60 \cdot \log [0.35 \cdot ([TPMP] \text{ added} - \text{external } [TPMP])/(0.001 \cdot \text{mg protein/ml} \cdot \text{external } [TPMP])]$.

Experimental approach to top-down elasticity analysis. Top-down elasticity analysis allows one to reduce the complexity of the system under investigation by grouping reactions into large blocks connected by a common intermediate (Fig. 1). This analysis involves measuring the kinetic responses of the blocks of reactions to changes in the common intermediate. By comparing the kinetic curves for the different reaction blocks in the presence and absence of the effector, it is easy to identify which blocks have a changed kinetic response to the intermediate and so can be identified as primary targets of the effector (14). In this work, the kinetic responses to changes in $\Delta\Psi$ of the reactions that consume $\Delta\Psi$ (proton leak and phosphorylating system) and those that produce it (substrate oxidation pathway) were measured by titrating with inhibitors which are very specific and have primary sites of action within the mitochondrial respiratory system (oligomycin, an inhibitor of ATP synthase, and malonate, an inhibitor of succinate dehydrogenase) (25). In each titration, mitochondria were incubated in the above medium for 3 min before the addition of 5 μM $TPMP^+$ to calibrate the $TPMP^+$ electrode. Respiration was then initiated by addition of 10 mM sodium succinate. As soon as the steady state was reached, the titration was performed by successive additions of the inhibitors.

If the activity of the substrate oxidation pathway is titrated with malonate (up to 5 mM) in the presence of oligomycin (2 μg/mg of protein) to prevent ATP synthesis, the resulting titration curve of $\Delta\Psi$ against respiration rate represents the kinetic response of the proton leak to changes in $\Delta\Psi$ (20, 25).

Moreover, if the titration with malonate (up to 5 mM) is repeated in state 3 (by adding 1 mM ADP in the absence of oligomycin) and the values are then corrected for the amount of oxygen required to balance the rate of proton leakage at each $\Delta\Psi$ measured, the resulting titration curve reflects the kinetic response of the phosphorylating system to $\Delta\Psi$ (20, 25).

Finally, if we manipulate the ATP synthesis capacity starting from state 3 (1 mM ADP) by titrating with oligomycin up to 2 μg/mg protein, the relationship between the rate of respiration and $\Delta\Psi$ will reflect the kinetic response of substrate oxidation pathway to $\Delta\Psi$ (20, 25).

Measurement of Q-2 redox states and determination of the kinetic responses of Q-2 reducers and Q-2 oxidizers to changes in Q-2 redox state. In order to evaluate the kinetics of Q-2 reducers and Q-2 oxidizers, the Q-2 redox state and respiration rate were measured in the incubation medium supplemented with 10 μM Q-2 and 5 μM rotenone. Q-2 is a short analogue of the endogenous ubiquinone 10, can interact directly and rapidly with complex I, II, and III at their quinone binding sites (26, 27), and thus may be used to estimate the redox state of the quinone pool between these complexes (22, 24). The redox state of Q-2 was measured in a Perkin–Elmer 557 dual-wavelength spectrophotometer by using the wavelength pair 290 and 275 nm. The signal corresponding to fully oxidized Q-2 was found by adding excess uncoupler (1 μM FCCP) in the absence of succinate, and the signal corresponding to fully reduced Q-2 was found in the presence of succinate (10 mM) by addition of excess antimycin (1 μM), an inhibitor of complex III.

The kinetic responses of the Q-2 reducers and oxidizers to changes in Q-2 redox state were measured by titrating the respiration rate as follows. Mitochondria (0.5 mg/ml) were incubated at 30°C in 3 ml of medium supplemented with Q-2, rotenone, and FCCP (at the concentrations reported above) and vigorously stirred to obtain the steady state for the fully oxidized Q-2. Then succinate (10 mM) was added to start the respiration and to partially reduce the Q-2. The kinetic responses of Q-2 reducers and Q-2 oxidizers to Q-2 redox state were determined by titrating with myxothiazol (up to 0.016 μM) or malonate (up to 1 mM), respectively. In each case the fully reduced Q-2 signal was obtained at the end of the titration by the addition of excess antimycin (1 μM). These titrations were performed by recording oxygen consumption and Q-2 redox state in parallel experiments.

Elasticity coefficients of Q-2 reducers and Q-2 oxidizers to Q-2 redox state (defined as the variation in the flux due to an infinitesimal change in the activity of the enzyme block) were measured considering the reciprocal value of the regression line slope interpolating the points of myxothiazol and malonate titration, respectively (14, 28, 29). Flux control coefficients (which quantify the control exerted by each step over a pathway flux) of Q-2 reducers and oxidizers were calculated using the above elasticity coefficients on the basis of the flux control summation and connectivity theorems (14, 28, 29).

Determination of cytochrome content. The cytochrome content of both sets of mitochondria was determined by recording the reduced–oxidized difference spectrum of the cytochromes using a double-beam spectrophotometer (30). Mitochondria were suspended to a concentration of 2 mg/ml in the standard medium supplemented with rotenone (5 μM) and placed in the reference and sample cuvettes stirred at 30°C. After addition of FCCP (1 μM) to both cuvettes, the oxidized

cytochrome spectra from 500 to 650 nm were recorded as baseline. Then the reduced minus oxidized spectrum was obtained by adding a few grains of sodium dithionite to the sample cuvette to completely reduce the cytochromes and by recording after 2 min the spectrum of the reduced-sample cuvette versus the oxidized-reference cuvette from 500 to 650 nm. The differences in absorbancy at the following pairs of wavelengths (nm) were then taken from each curve: 550/535 (cytochrome c), 554/540 (cytochrome c_1), 563/577 (cytochrome b), and 605/630 (cytochrome a-a_3). The relative amounts of the cytochromes were calculated by measuring the absorbances at the given wavelength pairs and substituting them in the equations described by Williams (30).

Statistics and materials. All statistical analyses were performed by the two-tailed Student *t* test; *P* values less than 0.05 were considered significant. Oligomycin, nigericin, malonate, and ADP were obtained from Sigma Chemical Co. (Poole, Dorset, UK). All other reagents used were of the highest purity commercially available.

RESULTS

State 3 and state 4 oxygen consumption was measured in liver mitochondria isolated from 25- and 60-day-old rats using succinate as substrate. State 3 oxygen consumption significantly increased in 60- compared to 25-day-old rats (state 3: 184.6 ± 9.8 and 155.5 ± 7.4 nmol O/min · mg protein in 60- and 25-day-old rats, respectively, $n = 7$, $P < 0.05$), while no significant difference was found in state 4 respiration (state 4: 64.0 ± 6.3 and 58.4 ± 5.5 nmol O/min · mg protein in 60- and 25-day-old rats, respectively).

In order to identify which component of the respiration system was responsible for the increased mitochondrial respiration rate in 60-day-old rats, the kinetic responses to $\Delta\Psi$ of proton leak (Fig. 3A), phosphorylating system (Fig. 3B), and substrate oxidation pathway (Fig. 4) were measured in both sets of mitochondria using succinate as substrate. Figure 3A shows no difference in the kinetic responses of proton leak, while Fig. 3B shows a slightly decreased kinetic response of phosphorylating system to $\Delta\Psi$ in isolated mitochondria from 60- compared to 25-day-old rats. In fact, at any given $\Delta\Psi$, the respiration rate is lower in mitochondria from 60-day-old rats than in mitochondria from 25-day-old rats. On the other hand, Fig. 4 shows that the kinetic response of substrate oxidation pathway to $\Delta\Psi$ is significantly increased in mitochondria from 60-day-old rats compared to those from 25-day-old rats. In fact, the plot of mitochondrial oxygen rate against $\Delta\Psi$, obtained with mitochondria from 60-day-old rats, lies above the plot obtained with mitochondria from 25-day-old rats, such that, at any given $\Delta\Psi$, the oxygen consumption rate is higher in 60-day-old rats than in 25-day-old rats. This result indicates that the substrate oxidation pathway is stimulated in mitochondria from 60-day-old rats using succinate as substrate.

Taking into account this result, the top-down elasticity analysis has been applied to the substrate oxidation pathway, which has been considered constituted by Q-2 reducers and Q-2 oxidizers with the Q-2 redox state (expressed as % of Q-2 reduced) as common intermediate. As shown in Fig. 5, uncoupled mitochondria respiring succinate in the presence of Q-2 exhibit a higher respiration rate (+37%) in 60-day-old rats than in 25-day-old rats, while no variation was found in the Q-2 redox state (about 22% of Q-2 reduced) in both sets of mitochondria. Figure 5 shows the variations in Q-2 redox state and in respiration rate during malonate and myxothiazol titrations in order to determine, respectively, the kinetic responses of Q-2 oxidizers and reducers. The kinetic response of Q-2 oxidizers is stimulated in mitochondria from 60-day-old rats; in fact, at any Q-2 redox state, the respiration rate is higher in mitochondria from 60- than from 25-day-old rats. From

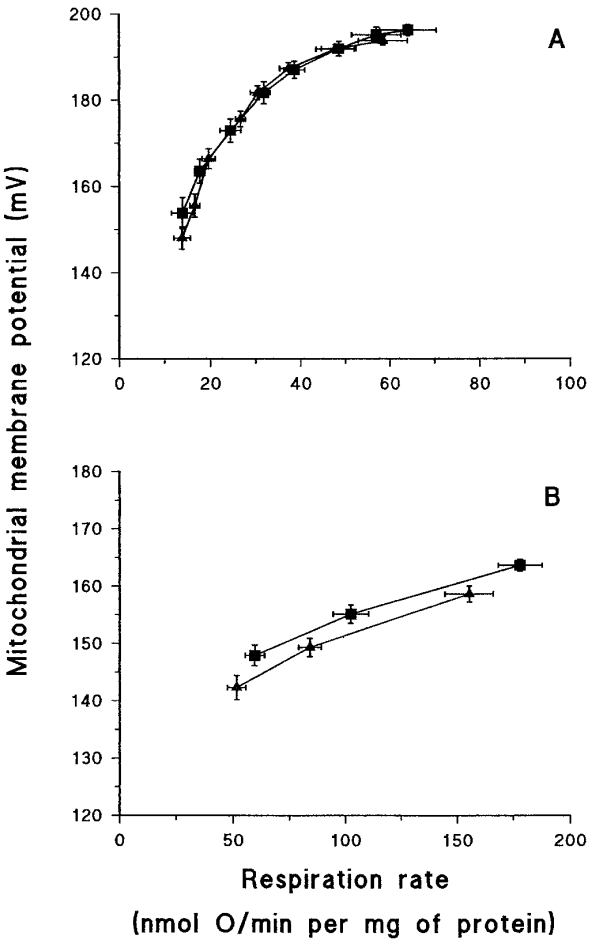

FIG. 3. Comparison of the kinetic response of $\Delta\Psi$ consumers to $\Delta\Psi$ in liver mitochondria isolated from 25-day-old (▲) and 60-day-old (■) rats. (A) Kinetic response of the mitochondrial proton leak to $\Delta\Psi$: Malonate titration (up to 5 mM) of oligomycin inhibited respiration (2 μg/mg of protein). (B) Kinetic response of the phosphorylating system to $\Delta\Psi$: Malonate titration (up to 5 mM) of state 3 (1 mM ADP) in the absence of oligomycin, minus the malonate titration in the presence of oligomycin. Each point is the mean ± SE of seven different experiments, each carried out in triplicate.

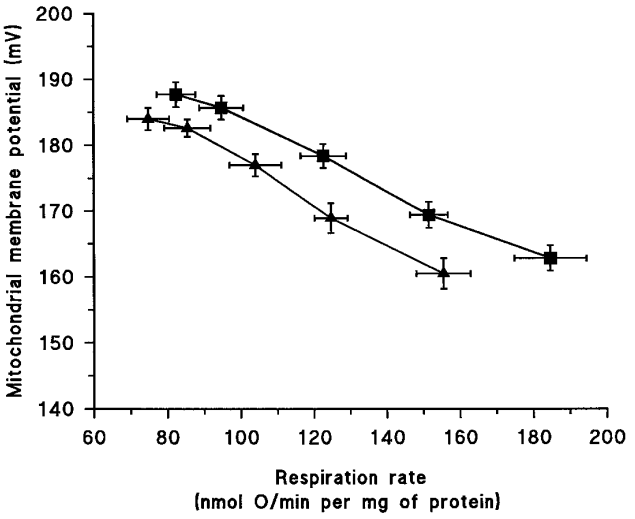

FIG. 4. Comparison of the kinetic response of $\Delta\Psi$ producers (substrate oxidation pathway) to $\Delta\Psi$ in liver mitochondria isolated from 25-day-old (▲) and 60-day-old (■) rats. Oligomycin titration (up to 2 μg/mg protein) starting from state 3 (1 mM ADP). Each point is the mean ± SE of seven different experiments, each carried out in triplicate.

the reciprocal value of the slope of the regression line interpolating points of the malonate titrations, it is possible to evaluate the elasticity coefficients of Q-2 oxidizers: 5.55 for 60- and 4.16 for 25-day-old rats.

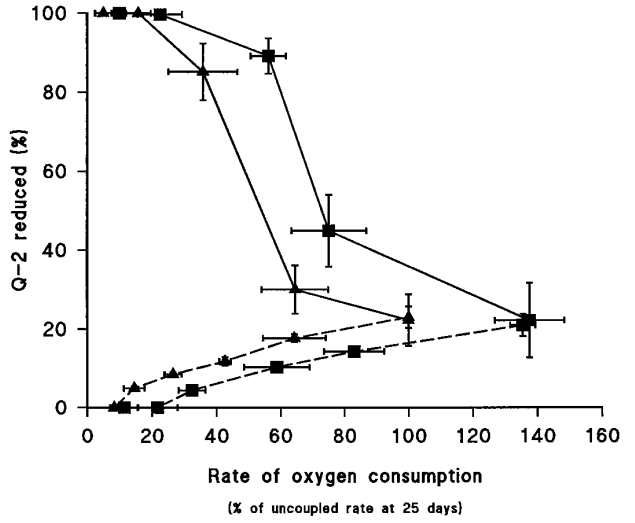

FIG. 5. Comparison of the kinetic response of Q-2 reducers and Q-2 oxidizers to Q-2 redox state in uncoupled liver mitochondria isolated from 25-day-old (▲) and 60-day-old (■) rats. Rat liver mitochondria (0.5 mg/ml) were incubated at 30°C in the medium supplemented with Q-2 (10 μM), rotenone (5 μM), FCCP (1 μM), and succinate (10 mM). Respiration was then titrated either with malonate up to 1 mM (dashed line, kinetic response of Q-2 oxidizers to Q-2 reduction) or with myxothiazol up to 0.016 μM (solid line, kinetic response of Q-2 reducers to Q-2 reduction). Each point is the mean ± SE of three different experiments, each carried out in duplicate.

TABLE I

Mitochondrial Cytochrome Content

	25-day-old rats	60-day-old rats
Complex III (cytochromes b, c_1)	0.217 ± 0.033	0.338 ± 0.045*
Cytochrome c	0.152 ± 0.024	0.222 ± 0.020*
Complex IV (cytochromes a, a_3)	0.212 ± 0.034	0.305 ± 0.007*

Note. Values are expressed as nmol/mg protein and are the means ± SE of four different experiments, each carried out in triplicate.
* $P < 0.05$ compared to 25-day-old rats.

The kinetic response of Q-2 reducers is also stimulated in mitochondria from 60-day-old rats; in fact, at any respiration rate, the Q-2 redox state is greater in mitochondria from 60-day-old rats than in those from 25-day-old rats. From Fig. 5, the elasticity coefficients of Q-2 reducers were 1.49 for 60- and 1.05 for 25-day-old rats.

From the elasticity summation and connectivity theorems, the values of the control coefficients of Q-2 oxidizers and Q-2 reducers were 0.21 and 0.79, respectively, for both sets of mitochondria.

In order to study thoroughly the variation in the kinetics of Q-2 oxidizers, the cytochrome contents of both sets of mitochondria were measured (Table I). The results show that all three complexes (complex III, cytochrome c, and complex IV) significantly increased by 56, 46, and 44%, respectively, in mitochondria isolated from 60-day-old rats compared to those from 25-day-old rats.

DISCUSSION

In the present study, the hepatic mitochondrial respiratory system exhibited significant changes in the transition from weaning to adulthood in the rat.

We have measured hepatic mitochondrial respiratory rates in liver mitochondria isolated from 25- and 60-day-old rats. The substrate utilized was succinate, which is a FAD-linked substrate and enters the electron transport chain at complex II. The significantly higher oxygen consumption found in 60-day-old rats could be due to variation in the substrate oxidation pathway, the phosphorylating system, and/or the proton leakage. In order to verify which of the above subsystems was changed in mitochondria from 60-day-old rats, we investigated the kinetic responses of proton leak, phosphorylating system, and substrate oxidation pathway to $\Delta\Psi$ in isolated liver mitochondria from 25- and 60-day-old rats using top-down elasticity analysis (14–20).

In agreement with the increased respiration rate, Fig. 4 shows a stimulation in the rate of the substrate

oxidation reactions at a given $\Delta\Psi$ in mitochondria from 60-day-old rats. On the other hand, the plot of the kinetic response of the proton leak to $\Delta\Psi$ (Fig. 3A) shows that there is no difference in the rate of the proton leak pathway at any given $\Delta\Psi$ between mitochondria isolated from 25- and from 60-day-old rats, according to the unchanged state 4 respiration rate found in this paper. Interestingly, despite the increase in state 3 respiration rate found in 60-day-old rats, Fig. 3B shows a slight decrease in the kinetic response of the phosphorylating system to $\Delta\Psi$ in these rats. Hence, the increased capacity of the substrate oxidation pathway to produce $\Delta\Psi$ is not matched by an increased capacity of the phosphorylating system activity and/or the proton leak to consume it, resulting in a higher $\Delta\Psi$ in 60- than in 25-day-old rats. Therefore, in 60-day-old rats a new state 3 steady state is reached in which higher respiration rates and $\Delta\Psi$ values are associated with an increased substrate oxidation pathway and with a decreased phosphorylating system activity. This decrease can be due to a decrease in the adenine nucleotide carrier, phosphate carrier, and/or ATP-synthase activity. It is interesting to note that a decline in the rate of mitochondrial ATP production in aging rats, probably due to a decreased ATP-synthase activity, has been reported in the literature (3, 13).

The increased kinetic response of $\Delta\Psi$ producers found in mitochondria from 60-day-old rats can be due to changes in the respiratory chain from complex II onward, substrate transport systems, and/or substrate dehydrogenases. To gain further insight into the mechanism by which the substrate oxidation pathway is stimulated in these rats, we applied the top-down approach to the substrate oxidation system, dividing it into blocks of processes around Q-2 as shown in Fig. 1. The analysis of the kinetic responses of the above blocks of reactions to changes in the Q-2 redox state showed that both Q-2 reducers and Q-2 oxidizers (Fig. 5) are stimulated in mitochondria from 60-day-old rats compared to those from 25-day-old rats. The flux control coefficients of Q-2 reducers (79%) and oxidizers (21%) remain at the same values in both sets of mitochondria, indicating that the flux control of either Q-2 reducers or Q-2 oxidizers over the substrate oxidation pathway does not change in the transition from weaning to adulthood. In addition, since there is an increased uncoupled respiration rate in mitochondria isolated from 60-day-old rats without any change in the intermediate Q-2 redox state (Fig. 5), on the basis of the proportional activation approach proposed in (31), it seems feasible that both subsystems of Q-2 reducers and oxidizers are activated to exactly the same extent in mitochondria isolated from 60-day-old rats. The increase in the activity of the Q-2 reducers could be due to an increased dicarboxylate carrier and/or to an altered activity of mitochondrial dehydrogenases (complex II). In agreement with this result, our previous work showed an increase (about 65%) in the activity of the succinate dehydrogenase in mitochondria from 60-day-old rats (32). On the other hand, the increased activity of Q-2 oxidizers could be due to changes in the respiratory chain from the complex III onward.

In order to verify which of the components of the Q-2 oxidizers was altered in the mitochondria from 60-day-old rats, we measured the cytochrome content of both sets of mitochondria. The results show that the content of all three cytochrome complexes increased by about 50% in mitochondria isolated from 60-day-old rats. This increased cytochrome content is not due to an increase in the mitochondrial membrane surface, since the proton leakage and the ATP turnovers do not increase. In addition, since there is an equal activation of Q-2 oxidizers and reducers, complex II and/or dicarboxylate carrier should be stimulated to the same extent (about 50%) as the cytochromes. Taking into account the extent of the increased succinate dehydrogenase activity previously found (32), there seems to be no reason to propose any increase in the dicarboxylate carrier.

In conclusion, it seems that the most important stages of development are associated with changes in liver activity. In fact, our results clearly indicate that in the transition from weaning to adulthood there are modifications in the hepatic mitochondrial compartment, namely, a stimulation in the substrate oxidation pathway activity and an increased cytochrome content. However, these increases are not matched by increased phosphorylating system activity. Taking together the above results, it can be suggested that in 60-day-old rats the liver metabolic efficiency of mitochondrial respiration declines, at least under conditions of extreme energy stress, i.e., when the requirement of ATP production is very high. Therefore, it seems feasible that in the transition from weaning to adulthood there is an impairment in the hepatic mitochondrial ability to adapt to the drastic increase in energy requirements.

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