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Body size effects on tissue metabolic rate and ischemia tolerance in neonatal rat and mouse hearts☆

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Abstract

Although the specific basal metabolic rate increases with decreasing body mass, newborn mammals are known to exhibit a higher tolerance to hypoxia/ischemia than adults. This is partly due to their ability to reduce energy demands in response to impaired supply. The so-called "hypoxic hypometabolism" might be explained as a temporary return to a prenatal metabolic state where the usual metabolic size relationship is suppressed and the fetus exhibits an "adult-like" specific metabolic rate. To study the interrelationship of body size, metabolic rate, and ischemia tolerance within and across species, both myocardial thin slices (to determine aerobic metabolic rates) and isolated non-perfused hearts (to assess ischemic "dying curves") from neonatal, juvenile, and adult rats were measured by microcalorimetry (2277 Thermal Activity Monitor, ThermoMetric, Sweden) and the results compared with earlier findings on mouse hearts. The aerobic tissue metabolic rates of myocardial samples from both species decreased with increasing body mass, according to the overall metabolic size relationship. Moreover, a slowing-down of the ischemic "dying curves" with decreasing body mass was found, reflecting the increasing hypoxia/ischemia tolerance. However, the amount of heat produced during ischemia turned out to be higher in neonatal rats than in juvenile mice, or in juvenile rats than in adult mice, respectively, despite nearly identical body and organ weights. The factor by which the ischemia tolerance of rats exceeds that of mice of comparable size, corresponds to the difference in specific basal metabolic rates to be expected between adult individuals of the same species. This is suggestive of a temporary return to an "adult-like" metabolic level underlying the elevated hypoxia/ischemia tolerance in neonatal and juvenile mammals.

Keywords: Neonate; Body size; Metabolic rate; Hypoxia tolerance; Calorimetry

1. Introduction

Following a general metabolic law, often referred to as Kleiber's rule, the specific (i.e. mass-related) basal metabolic rate of mammals decreases with increasing body size [1,2]. Although the exact cause of the "allometric" size relationship is not yet fully understood [3,4], it has been shown that under conditions of ischemia (interrupted blood perfusion), larger mammals undergo a slower breakdown in tissue heat output than smaller ones, according to their lower specific basal metabolic rate. When the metabolic rate of "large mammal" organs is further reduced (e.g. by induced hypothermia and/or by appropriate preservative solutions), they can even reach a metabolic level that is temporarily met by anaerobic glycolysis, thus resulting in an intermediate "plateau" of heat output [5,6].

In spite of the increasing specific basal metabolic rate with decreasing body mass, newborn mammals have long been known to exhibit a higher tolerance to hypoxia or ischemia than adult mammals [7,8]. Their elevated hypoxia tolerance which may be regarded as an adaptation to the risk of birth

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asphyxia, is due to a variety of factors including, among others, the lower metabolic demand of brain tissue [9–11], the energy-saving circulatory [12–14] and thermoregulatory [15–17] response pattern as well as ample muscle glycogen stores enhancing the efficiency of anaerobic glycolysis [18–20]. Moreover, another mechanism has recently been attracting increasing interest, namely, the ability of neonatal mammals to reduce their metabolic demand in response to impaired oxygen supply, without suffering from an oxygen debt [7,8,15,21,22]. This ability, which so far has only been known from invertebrates and lower vertebrates [23,24] and whose mechanisms are still a matter of debate [25,26], is called "hypoxic hypometabolism".

In an earlier microcalorimetric study on mouse hearts [27,28], we were able to show that the ischemic decline in heat output was much slower in neonatal than in juvenile or adult individuals, although the aerobic heat output rate was highest in neonatal tissue samples, according to the low body size. The "paradoxical" increase in ischemia tolerance (despite a higher tissue metabolic rate) was tentatively explained by a "left-shift" of the pO_2/metabolic rate relationship:

When measured under different oxygen tensions, neonatal tissue samples, at low pO_2, still exhibited the same metabolic rate as adult samples at high pO_2. To understand this observation, it is important to be aware of the fact that although the arterial pO_2 in the fetal circulation amounts to 3.3–4.0 kPa (25–30 mmHg) in contrast to 12.0–13.3 kPa (90–100 mmHg) in the adult circulation, the fetus is known to exhibit similar metabolic rates as "an organ of its mother" [29–31]. Therefore, the metabolic compensation to be observed in neonatal tissue seems to enable the fetus to maintain an "adult-like" metabolic rate, despite the greatly restricted oxygen supply through the placenta.

The same adaptation might also underlie the postnatal increase in metabolic rate up to the level to be expected from body mass, as a consequence of the perinatal increase in oxygen tension. In the case of an oxygen lack, however, it seems to allow a temporary return to the feto-maternal ("adult-like") metabolic level, thus explaining at least part of the "hypoxic hypometabolism" to be found in mammalian neonates (Fig. 1).

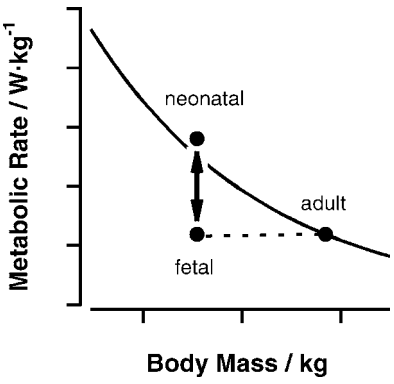

Fig. 1. Schematic illustration of the postnatal metabolic increase from the feto-maternal ("adult-like") level up to the turnover rate to be expected from the overall size relationship (Kleiber's rule). A temporary reversal of this metabolic increase might underlie the "hypoxic hypometabolism" to be observed in newborn mammals.

Given this hypothesis, the ischemic breakdown of metabolism in newborn mammals of differing size would be expected to relate to the adult rather than to the neonate's own metabolic rates. Therefore, we decided to reproduce the above-mentioned mouse experiments in another species of slightly differing size and to subject the data to a comparative analysis.

2. Experimental

To relate organ ischemia tolerance to tissue metabolic rate, two different preparations of heart tissue from neonatal (day 0), juvenile (day 13/14/15), and adult Wistar rats of each sex were studied by microcalorimetry (2277 Thermal Activity Monitor, ThermoMetric, Järfälla, Sweden):

(i) To measure aerobic tissue metabolic rates, 300 μm samples of left-ventricular myocardium were cut using a tissue slicer (752 M Vibroslice, Campden Instruments, Leicester, UK) and put into air-saturated Hanks' solution (balanced salts, glucose content 1 g/l, $pO_2 \approx 20$ kPa [150 mmHg]) at 25 °C. The incubation of thin slices in an aerated nutritional solution at lowered temperature ensured that mostly aerobic conditions, according to Warburg's formula [32], were maintained with the resulting heat output records remaining fairly stable. Tissue heat output rates were read 90 min after the start of measurement.

(ii) To determine the hypoxia/ischemia tolerance, whole organs or large ventricular samples, respectively, were put into Ringer's solution at 25 °C. Due to their large size (curtailing oxygen supply by diffusion from the surroundings), these were mostly ischemic so that the microcalorimetric recordings reflected the subsequent decline in metabolic rate. Nevertheless, the incubation temperature of 25 °C was chosen to prevent the "dying curves" from being too rapid for proper analysis. To quantify the rate of metabolic decline, the total amount of heat liberated during a 3 h interval (from 30 to 210 min after the onset of ischemia, corresponding to 0 to 180 min after the onset of measurement) was calculated. A high total heat output means a slow "dying curve", and vice versa.

Details of the microcalorimetric technique are described elsewhere [5,33]. Briefly, the 2277 Thermal Activity Monitor (TAM) is an isothermal heat conduction calorimeter with four measuring cylinders, each equipped with a measuring and a reference chamber and an electrical calibration unit. Experiments were done in the "batch mode", i.e. the tissue samples were put into 4 ml stainless steel ampoules, which were filled with 3.5 ml of the appropriate incubation solution (as were the reference ampoules). To allow a thermal

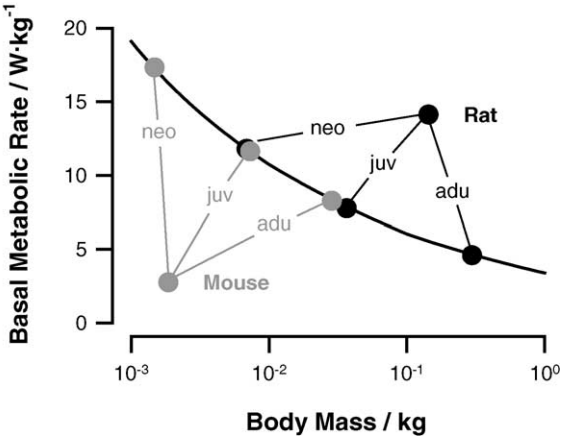

Fig. 2. Overlap in body size and metabolic rate between the species studied in this investigation. Due to their nearly identical body weights, also the specific basal metabolic rates (according to the overall metabolic size relationship) will be similar in neonatal rats to those in juvenile mice, and in juvenile rats to those in adult mice, respectively.

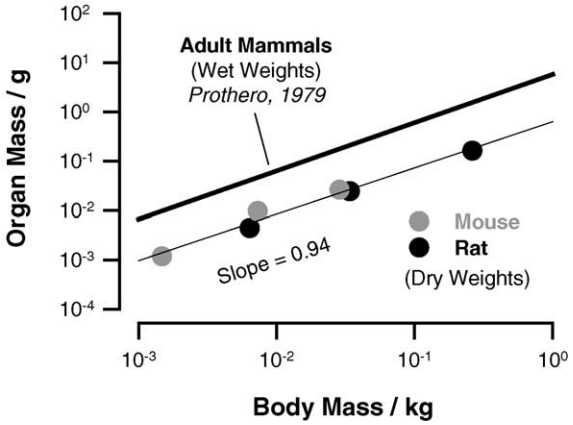

Fig. 3. Dry weights of neonatal, juvenile, and adult mouse and rat hearts as related to body size. The data fit an isometric (directly proportional) size relationship paralleling that given by Prothero [34] for the wet weights of mammalian hearts across species.

equilibration period of 15–20 min before lowering the ampoules into the measuring chambers, the measurements were started 30 min after the excision of the organs. At the end of the calorimetric experiments, all samples were removed from the ampoules and put into a drying chamber (Memmert, Schwabach, Germany) at 90 °C for 24 h. Dry weights were determined using a precision balance (2001 MP2, Sartorius, Göttingen, Germany).

The murine data referred to in this study were published in an earlier paper [28]. The heart was chosen as the experimental organ since in earlier investigations, it had proven to widely follow the whole body metabolic size relationship, apparently due to its major role in energy supply [5,6,27,28]. The rat was selected for comparison with regard to the "size overlap" between the two species, allowing a distinction between the metabolic effects of body mass and of developmental stage (Fig. 2). Painless sacrifice was performed according to the guidelines for animal research and prescriptively reported to the regulatory authorities.

3. Results and discussion

The mean dry weights of both rat and mouse hearts from different developmental stages are shown in Fig. 3. Remarkably, when plotted on log scales, they fit a line with a slope of roughly 1.0, indicating an "isometric" (directly proportional) body size relationship that parallels the one known for wet weights of adult mammal hearts across species [34]. Thus, they follow a general rule according to which relative organ masses remain more or less constant, independent of varying metabolic demands resulting from different body masses or developmental stages [2,35]. In the case of the heart, this means that also the specific (mass-related) stroke volume is fairly independent of body size while the increase in specific metabolic rate with decreasing body size is reflected by a corresponding increase in beat frequency [36].

In view of the increasing functional demands to be met with decreasing body size, it is not surprising that the metabolic rate of myocardial tissue varies accordingly. This is reflected by the specific heat output rates of thin slices, amounting to 3.59 ± 1.79 (n = 12), 2.22 ± 0.53 (n = 10), and 2.08 ± 0.54 (n = 14) mW g_{dw}^{-1} (mean ± S.D., dw = dry weight) in neonatal, juvenile, and adult samples, respectively. When these are plotted along with the corresponding mouse heart data (Fig. 4), they decrease with body mass to the power of −0.25 and, thus, exactly parallel Kleiber's rule for the size relationship of specific basal metabolic rate in adult mammals. Hence, they correspond to the findings of Krebs [37] on the respiration rate of tissue samples from a variety of adult mammals. On the other hand, this result seems to contradict earlier reports on a slow postnatal increase in metabolic

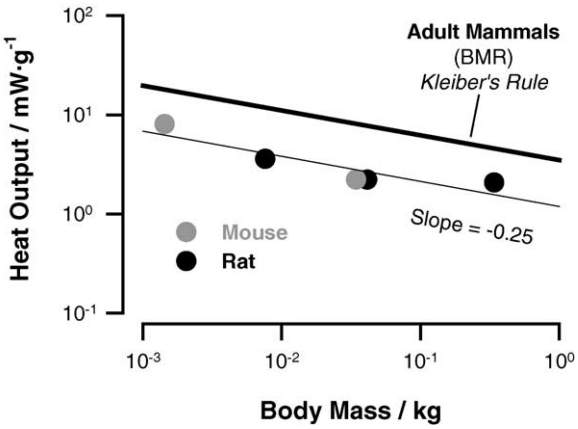

Fig. 4. Aerobic tissue metabolic rates (per gram dry weight) of neonatal, juvenile, and adult mouse and rat hearts as related to body size. The data fit an allometric size relationship (indicating a decrease in specific metabolic rate with increasing body size) paralleling that known for the specific basal metabolic rates (BMR, per gram wet weight) of mammals across species (Kleiber's rule).

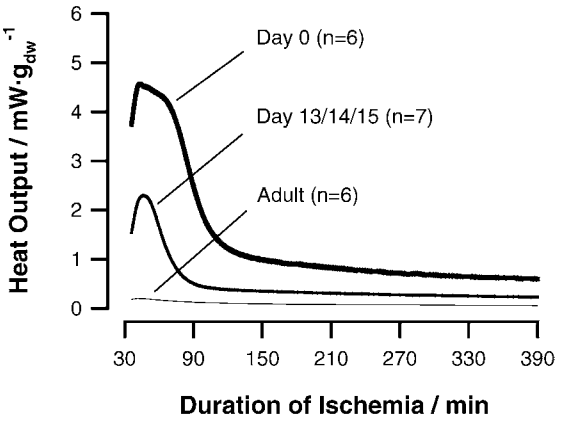

Fig. 5. Ischemic heat output rates (per gram dry weight, dw) of neonatal, juvenile, and adult rat hearts as recorded by microcalorimetry (the initial rise in heat output seen in the neonatal and juvenile groups reflects the increase in the calorimetric signal occurring after the introduction of the samples into the measuring chambers). The "dying curve" is much slower in neonatal than in juvenile or even in adult hearts where the ischemic decline in metabolic rate occurs so fast that the final baseline is already attained at the start of measurement.

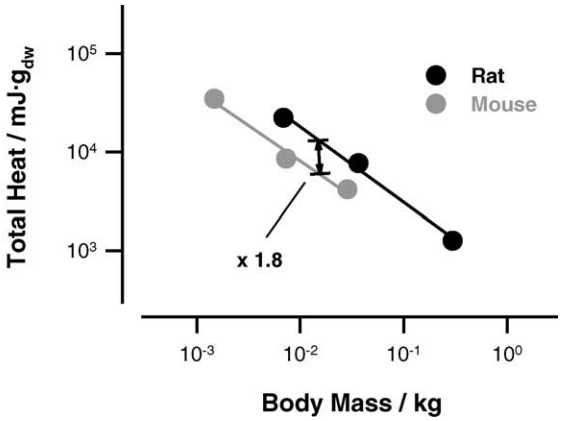

Fig. 6. Total amount of heat (per gram dry weight, dw) produced by ischemic hearts from neonatal, juvenile, and adult mice and rats. The data points represent the specific sum of heat liberated within the time interval from 30 to 210 min after the onset ischemia (or from 0 to 180 min after the onset of measurement, respectively). When plotted against body mass, those integrated heat output rates turn out to be higher (i.e. the "dying curves" are correspondingly slower) in rat than in mouse organs with the difference amounting to a factor of 1.8.

rate, lasting up to three weeks, in newborn rats [35,38,39]. To resolve this apparent contradiction, it should be taken into account that the myocardial slices were incubated in fully aerated nutritional solution and were thus exposed to oxygen tensions which by far exceed those to be expected in the arterial blood of intact newborn individuals. Provided that the metabolic rate of neonatal tissue depends on oxygen supply (cf. below), the in vitro heat output rate of neonatal tissue might already reflect a metabolic level that has still not been attained in vivo.

This assumption is reinforced by the "paradoxical" increase in ischemia tolerance (despite the increase in aerobic tissue metabolic rate) that is reflected by the dramatic slowing-down in the "dying curves" with decreasing age and size (Fig. 5). This result which exactly corresponds to our earlier findings in mouse hearts [27,28], might be partly due to a reverse "crowding effect", i.e. to a better supply of physically dissolved oxygen (by diffusion from the incubation medium) to smaller than to larger organs [6,40]. This might also explain why the ischemic "dying curve" is somewhat faster in adult rat hearts than it has been found to be in adult mouse hearts. However, when the specific sum of heat produced during a definite interval of ischemia is related to body weight, it becomes obvious that, at any given body and organ weight, this is even higher in rat than in mouse hearts (Fig. 6). Obviously, under conditions of ischemia, more heat is produced in the neonatal rat heart than in the juvenile mouse heart, or in the juvenile rat heart than in the adult mouse heart, respectively, regardless of the nearly identical body and organ weights. Hence, the difference in ischemia tolerance appears to be related to the degree of maturity rather than to organ size per se.

This might be at least partly explained by tissue characteristics such as the higher glycogen stores in growing muscles (cf. above). Remarkably, however, the ratio by which the ischemic heat output of rat hearts exceeds that of mouse hearts, amounts to 1.8 and thus exactly corresponds to the ratio of specific basal metabolic rates in adult mice and rats, according to Kleiber's rule (Fig. 7). This is consistent with the initial hypothesis that under conditions of impaired energy supply, neonatal and juvenile mammals may return to their respective feto-maternal ("adult-like") metabolic levels and, thereby, differ in ischemia tolerance although the actual body and organ weights as well as the corresponding aerobic tissue metabolic rates would not explain such a difference.

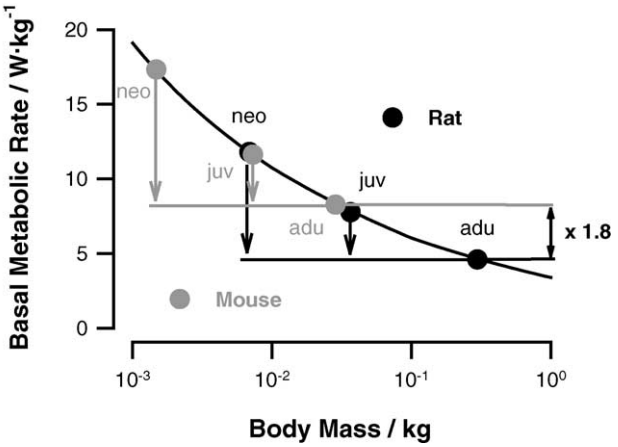

Fig. 7. Putative mechanism of "hypoxic hypometabolism" in neonatal and juvenile mammals. Provided the metabolic rate is turned down to an "adult-like" level (cf. Fig. 1), rats and mice should differ by a factor of 1.8, irrespective of comparable body and organ weights. This is in perfect accordance with the difference in ischemic tissue heat output rates found in this study (cf. Fig. 6).

4. Conclusions

In summary, the inter-species comparison of two mammals overlapping in size has shown that both organ weights and tissue metabolic rates fit an overall size relationship, independently of species and maturity. Moreover, in both species, an increasing ischemia tolerance with decreasing age and size is found, in apparent contradiction to the increasing aerobic tissue metabolic rates. However, the amount of heat liberated during ischemia, i.e. the velocity of the "dying curves", differs between species in spite of nearly identical body and organ weights. The ischemia tolerance is higher in the larger (rat) than in the smaller species (mouse), with the difference corresponding to the difference in specific basal metabolic rates between adult individuals. This result provides further support to the hypothesis that the "hypoxic hypometabolism" of neonatal mammals implies a temporary return to an "adult-like" metabolic level, which seems to govern ischemia tolerance even in individuals of much smaller size.

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