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BRAIN AND BODY OXYGEN REQUIREMENTS OF *GNATHONEMUS PETERSII*, A FISH WITH AN EXCEPTIONALLY LARGE BRAIN

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Summary

Vertebrates have repeatedly been noted for having remarkably constant ratios of brain to body O_2 consumption, the brain using 2–8 % of resting body O_2 consumption, suggesting that evolution has put strict limits on the energetic cost of brain function. Only man, with a value of 20 %, is an exception to this rule. However, the results presented here suggest that, in the electric fish *Gnathonemus petersii*, the brain is responsible for approximately 60 % of body O_2 consumption, a figure three times higher than that for any other vertebrate studied, including man. The exceptionally high energetic cost of the *G. petersii* brain appears to be a consequence both of the brain being very large and of the fish being ectothermic.

It was also found that *G. petersii* has a high ability to utilise O_2 at low levels. Thus, during falling [O_2], this species was found to maintain both its O_2 uptake and its electric discharge rate down to an ambient O_2 level of 0.8 mg l^{-1} (at 26 °C), although it was unable to tolerate an [O_2] below 0.3 mg l^{-1}. During severe hypoxia (<0.8 mg l^{-1}), *G. petersii* attempted to gulp air from the water surface. These results establish a new record for the energetic cost of a vertebrate brain and they show that the species possessing such a brain has a high capacity for utilising O_2 at very low ambient concentrations.

Key words: Mormyridae, *Gnathonemus petersii*, oxygen consumption, anoxia, hypoxia, energetics, brain size, electric fishes.

Introduction

The brain of man has hitherto been thought to account for the largest fraction of whole-body energy expenditure in vertebrates. The human brain consumes some 20 % of the O_2 taken up by the resting body (Crile, 1941; Mink *et al.* 1981). This has been put forward as the only gross physiological measure that makes the human brain unique among vertebrates, since its relative size *per se*, although corresponding to an impressive 2 % of body mass, is surpassed by that of several small mammals and birds (Crile and Quiring, 1940; Krompecher and Lipák, 1966). It has been pointed out that other vertebrates are remarkably uniform with respect to the ratio of brain to body O_2 consumption, which is of the order of 0.02–0.08, essentially regardless of body size (Crile, 1941; Mink *et al.* 1981). In fact, the only vertebrates known to possess brains consuming more than 10 % of total O_2 intake are some primates (11–13 %) and man (Mink *et al.* 1981). It is possible that the brain of the porpoise *Phocaena phocaena* also consumes a comparatively high fraction of the total O_2 inspired, since it constitutes 1.2 % of body mass (Crile and Quiring, 1940).

The uniqueness of the human brain with regard to its exceptionally high rate of energy use appears to have remained undisputed. However, in Africa, there is a group of weakly electric fishes, the elephant nose fishes (Mormyridae), that has been noted for having unusually large brains (Erdl, 1846), a character that is probably, at least in part, related to their ability to communicate by generating and perceiving electric fields (Hopkins, 1983; Nieuwenhuys and Nicholson, 1969). In contrast to man, it is the cerebellum, and not the telencephalon (cerebrum) that is greatly enlarged in these fishes, the structure being appropriately named the gigantocerebellum. From an evolutionary and ecophysiological point of view, the energetic cost of this brain may be more important than its size *per se*. As ectothermic vertebrates, these fish are expected to have low rates of whole-body O_2 consumption which could result in the brain comprising a relatively large fraction of the whole-body energy budget. However, brain and body O_2 consumption have never been measured in mormyrids. Moreover, since the vertebrate brain is normally very sensitive to low P_{O_2}, the hypoxia-tolerance of an animal with such a huge brain is of particular interest.

The aim of the present study was to measure brain and body O_2 consumption in the west African mormyrid *Gnathonemus petersii*, so that the ratio of brain to body O_2 consumption could be estimated. In addition, the ability of this fish to regulate O_2 consumption and electric pulse rate in response to falling O_2 levels, as well as its tolerance to severe hypoxia, were examined.

Materials and methods

Gnathonemus petersii Günther were obtained from a fish dealer in Uppsala, Sweden. They were held in an aquarium at 26 °C (16 fish in 250 l) and fed chironomid larvae daily until satiation, for at least 1 month before the experiments. All experiments were carried out at 26 °C.

Four experiments were performed on the 16 fish available. In the first experiment, body O_2 consumption was determined in four fish during decreasing O_2 levels in a closed respirometer. In the second experiment, the brains of four fish were removed and O_2 consumption of brain slices was determined. In the third experiment, five fish were exposed to more rapidly falling O_2 levels by bubbling the water with N_2 and their behaviour was observed. In a final group of three fish, body O_2 consumption and the discharge rate of the electric organ were determined simultaneously during falling O_2 levels in a closed respirometer.

Body O_2 consumption rates of *G. petersii* at different ambient O_2 concentrations were measured using closed respirometry in a 2 l respirometer as previously described (Nilsson, 1992). Each fish, which had not been fed for 24 h, was allowed to respire in the respirometer until the O_2 concentration reached approximately 0.3 mg l^{-1}, which took 780–1500 min (depending on fish size). The rate of O_2 consumption was calculated over 6 min intervals. After having been put into the respirometer, the fish initially showed a relatively high level of locomotor activity, causing rates of O_2 consumption to increase above resting values. In consequence, the first 200 min of the experiments were excluded from the calculations. The critical ambient O_2 level was determined as follows. A linear regression line was calculated from the O_2 consumption values measured at ambient O_2 levels above 1.5 mg l^{-1} (since no tendency for a break in the O_2 consumption curve was seen above that level; Fig. 1). The critical ambient O_2 level was assumed to correspond to the first data point that fell below this line if all subsequent values of O_2 consumption were also below the regression line.

The electric discharge of the fish was recorded using two stainless-steel rods (30 mm long, 3 mm diameter) introduced into the respirometer and connected to an a.c. amplifier. The discharge frequency (Fig. 2) was calculated from the time taken to record 4096 discharges. The frequencies recorded (generally 10–15 pulses s^{-1}) constituted average values over 4.5–7 min periods.

Brain slices (300 μm thick) were prepared from the whole lateral half of a brain using a Vibratome (Vibroslice 752; WPI, UK). Their O_2 consumption rates were determined (within 1 h of preparation) in a 1.6 ml thermostatted (26 °C) glass chamber equipped with a Clark O_2 electrode. Approximately 40 mg of randomly selected brain slices were used for each measurement. The slice preparation and O_2 consumption measurements were carried out in oxygenated brain-slice Ringer's solution prepared as described previously (Johansson *et al.* 1995).

Values are presented as means ± S.D.

Results

Table 1 shows that the brain of *G. petersii* constitutes 3.1 % of the body mass and that it is responsible for 60 % of whole-body O_2 consumption. The corresponding values for the human brain are approximately 2.3 % and 20 %, respectively, while those for other vertebrates are considerably lower. The estimated *in situ* values for O_2 consumption of the *G. petersii* brain and the brains of other fishes were obtained by multiplying brain-slice O_2 consumption by a factor of two (see Discussion).

Whole-body O_2 consumption rates measured during decreasing ambient P_{O_2} (Figs 1, 2) showed that the critical O_2 level for steady rates of O_2 consumption was 0.77±0.10 mg O_2 l^{-1} (N=7), i.e. an O_2 saturation of approximately 10 % at 26 °C. At a normoxic O_2 level in water (5 mg l^{-1}), the rate of O_2 consumption of the fish was 0.102±0.020 mg g^{-1} h^{-1} (Table 1; Figs 1, 2).

In a separate experiment, five fish were put in a 5 l aquarium in which the O_2 content was lowered to less than 0.3 mg l^{-1} within 2 h by bubbling with N_2. When the O_2 concentration fell below 0.8 mg l^{-1}, the fish started to gulp air (in this case N_2) from the surface, clearly an attempt to increase their O_2 uptake. Finally, when the O_2 content fell below 0.3 mg l^{-1}, the experiment had to be abandoned, since within 14–18 min all fish had problems with maintaining an upright body posture, clearly indicating an inability to tolerate such a low ambient O_2 concentration.

Fig. 2 shows electric discharge frequencies and O_2 consumption rates of three individuals in response to falling ambient O_2 levels. Both the electric activity and the O_2 consumption remained relatively constant down to the same

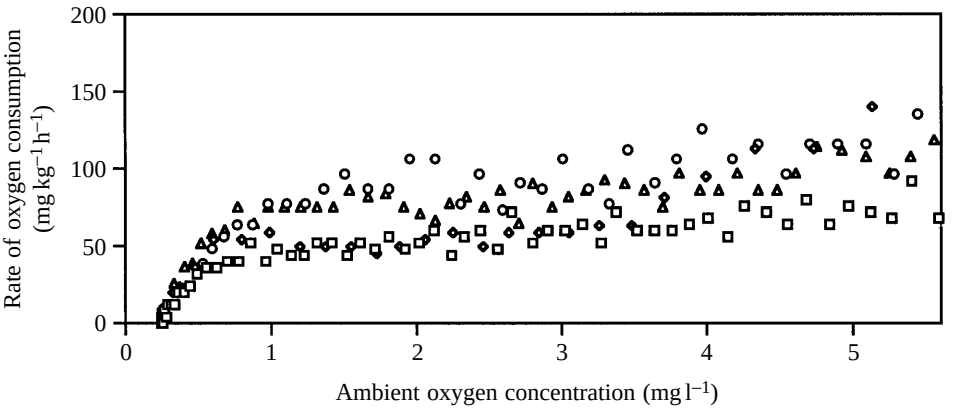

Fig. 1. O_2 consumption rate of *Gnathonemus petersii* at different water O_2 levels measured using closed respirometry. Different symbols are used to identify four individuals.

critical O_2 level ($0.82\pm0.05\,mg\,l^{-1}$ and $0.77\pm0.1\,mg\,l^{-1}$, respectively).

Discussion

Brain and body O_2 consumption rates

The brain of the mormyrid fish *G. petersii* consumes a larger fraction, i.e. approximately 60 %, of the whole-body O_2 intake than any other vertebrate brain studied, including the human brain, which had been thought to hold the record in this respect. The *in situ* O_2 consumption of the *G. petersii* brain (Table 1) was estimated by multiplying the *in vitro* values of brain slices by a factor of two. This was done because brain slices are energetically depressed, showing very low spontaneous electric activity. Brain slices from mammals consume only about half of the O_2 consumed by the intact brain (McIlwain and Bachelard, 1971; Mink *et al.* 1981; Lipton and Whittingham, 1984). The small size of *G. petersii* did not allow the catheterization of blood vessels necessary for measuring O_2 consumption of the brain *in situ*.

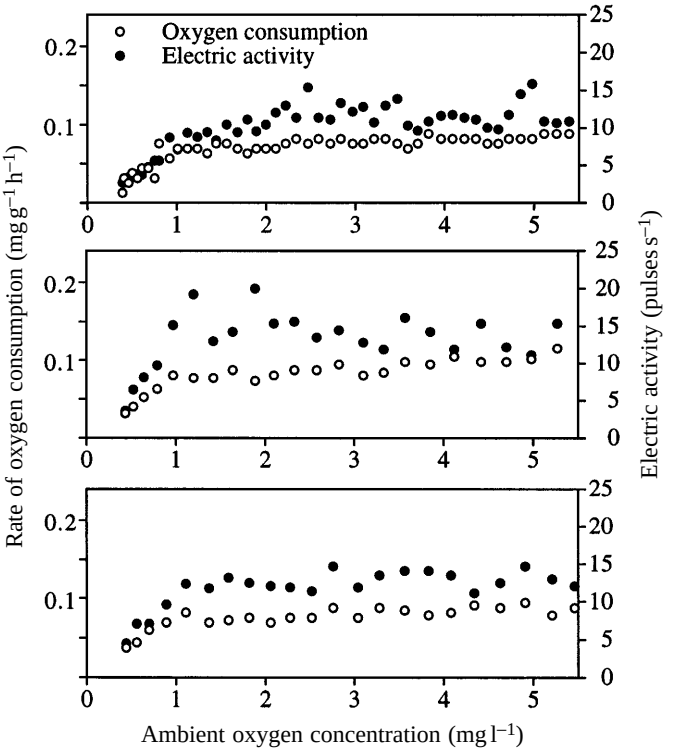

Fig. 2. *Gnathonemus petersii* electric discharge frequencies and O_2 consumption rates recorded simultaneously at different water O_2 levels. Each graph is for a different individual.

Whereas total energy expenditures of ectothermic vertebrates (Fig. 3B) are about 1/13 of those of endotherms at the same temperature and body size, mass-specific energy expenditures of the brains of ectothermic and endothermic vertebrates are much more similar (Fig. 3A). The average brain O_2 consumption rate of the three ectothermic vertebrates shown in Fig. 3A (derived from Table 1) is $4.95\,mg\,g^{-1}\,h^{-1}$ (at 37 °C), which is not much lower than the $7.74\,mg\,g^{-1}\,h^{-1}$ expected for an endothermic brain of the same average size (deduced from the regression line for endothermic brains in Fig. 3A). In fact, the O_2 consumption rate of the rainbow trout brain lies on the regression line for endothermic brains. Thus, with regard to the whole-body energy budget, it is comparatively more expensive for an ectothermic vertebrate to have a large brain. This may be a reason why most ectothermic vertebrates have relatively small brains, resulting in the strikingly constant ratio of brain to body O_2 consumption found for both ectothermic and endothermic vertebrates. Consequently, the fact that *G. petersii* is an ectothermic vertebrate and has such a huge brain makes this organ an exceptionally expensive part of the whole body.

Table 1. *Brain mass, body mass and O_2 consumption of* Gnathonemus petersii *and some other vertebrates*

Species	Temperature (°C)	Body mass (g)	Brain mass (g)	Mass ratio, brain/body	Body rate of O_2 consumption (mg g^{-1} h^{-1})	Brain slice rate of O_2 consumption (mg g^{-1} h^{-1})	Estimated rate of brain O_2 consumption *in situ* (mg g^{-1} h^{-1})	O_2 consumption ratio, brain/body
G. petersii	26	5.85±1.84	0.177±0.050	0.0309±0.0034	0.102±0.020	1.01±0.06	2.02±0.12	0.600±0.074
Carassius	20	10	0.100	0.0100	0.099	0.36	0.72	0.072
Salmo/Salvelinus	20	50	0.150	0.0030	0.160	1.10	2.20	0.041
Rattus	37	278	2.3	0.0083	1.045	3.07	6.02	0.048
Man	37	54 333	1274	0.0234	0.303		2.61	0.202

Values for *Salmo/Salvelinus* are from Peterson and Anderson (1969), except for body O_2 consumption which is from Beamish (1964).

Values for *Carassius* (*C. auratus* and *C. carassius*) are estimates from calorimetric measurements compiled or measured by Johansson *et al.* (1995).

Values for man and rat (*Rattus*) are those compiled by Mink *et al.* (1981), except for the rat brain slice data which are from Benjamin and Verjee (1980).

The data for *G. petersii* are means ± S.D. from seven (body O_2 consumption), four (brain slice O_2 consumption) or 12 (mass) animals.

The estimates of brain O_2 consumption *in situ* for the fishes were obtained by multiplying the brain slice values by a factor of two (see Discussion).

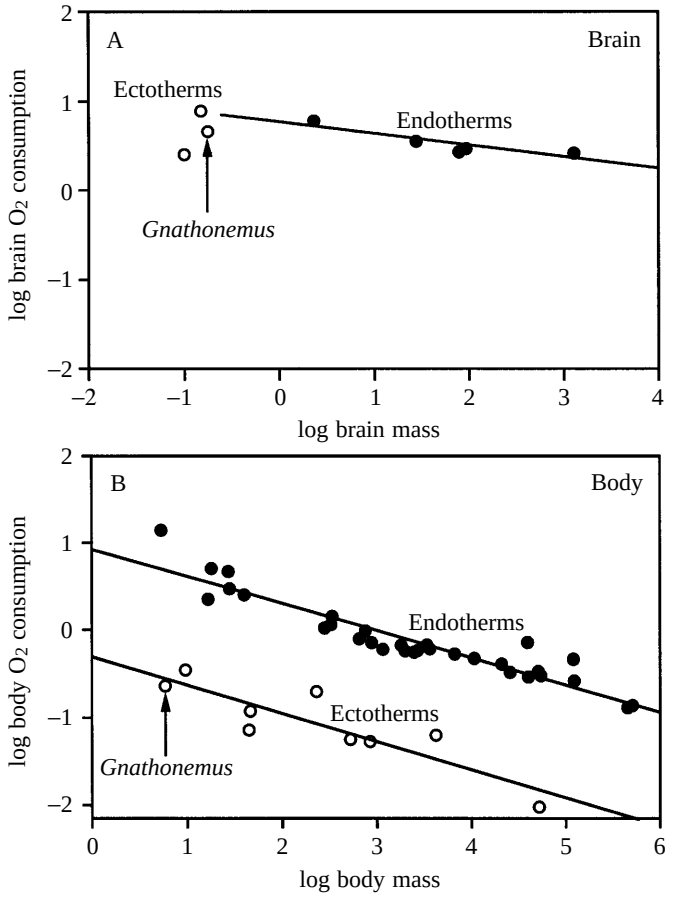

Fig. 3. Brain (A) and standard body (B) O_2 consumption rates in relation to mass in endothermic and ectothermic vertebrates (logarithmic scales). Note that the whole-body O_2 consumption rates are approximately 13 times higher in endotherms than in ectotherms, while there seems to be little or no difference between the O_2 consumption rates of endothermic and ectothermic brains. All values are estimates for 37 °C (assuming a Q_{10} of 2.1). Values for ectothermic brains are from Table 1. All other data (except for *Gnathonemus petersii*) are from measurements compiled by Mink *et al.* (1981). The equations for the lines and Pearson's correlation coefficients (r) are as follows. $y=0.767-0.136x$ ($r=-0.901$, $P=0.037$) for endothermic brains; $y=0.921-0.309x$ ($r=-0.938$, $P<0.0001$) for endothermic bodies; $y=-0.177-0.357x$ ($r=-0.86$, $P=0.0029$) for ectothermic bodies; where y is log O_2 consumption (in $mg\,O_2\,g^{-1}\,h^{-1}$) and x is log mass (in g).

As mentioned above, there are several small endothermic vertebrates, notably bats, mice, swallows, crows and sparrows, that have brains which make up 2.6–3.7 % of the body mass (Crile and Quiring, 1940; Mink *et al.* 1981). However, the very high whole-body metabolic rates of these small endotherms make their brains comparatively less costly (using only 4–8 % of the body energy budget), because in vertebrates the scaling factor of the mass-specific rate of energy expenditure is much lower for the brain than for the whole body (Fig. 3; Mink *et al.* 1981; Schmidt-Nielsen, 1984).

The Q_{10} for the rate of O_2 consumption of brain tissue is about 2.1 in vertebrates (Mink *et al.* 1981), suggesting that the specific rate of O_2 consumption of the *G. petersii* brain at 37 °C would be $4.57\,mg\,g^{-1}\,h^{-1}$. This is similar to the value of $6.02\,mg\,g^{-1}\,h^{-1}$ given for the rat brain by Nilsson and Siesjö (1976) and it is higher than the oxygen consumption of the human brain ($2.61\,mg\,g^{-1}\,h^{-1}$). The low value of the latter reflects the slightly negative scaling exponent of the metabolic rate of the brain. Among fishes, the brain of *G. petersii* does not have a uniquely high mass-specific rate of O_2 consumption; the value reported in this study was lower than the *in situ* value of $2.20\,mg\,g^{-1}\,h^{-1}$ at 20 °C estimated from previous brain slice measurements in salmonids (Table 1).

The reason why ectothermic brains do not differ much from endothermic brains with regard to the rate of energy utilisation is probably related to the fact that ionic leakage, which occurs at much higher rates and is largely coupled to heat production in endothermic vertebrates (Hulbert and Else, 1990), plays only a minor role in the energy expenditure of the brain. Ion movements involved in electric and synaptic activity are responsible for the largest portion of the energy used by the brain (Erecinska and Silver, 1989), and in this respect there is probably little difference between endothermic and ectothermic brains. Consequently, when exposed to anoxia, rainbow trout brain ion homeostasis becomes disrupted due to energy failure as fast as does that of the mammalian brain (Nilsson *et al.* 1993).

Hypoxia tolerance

The striking hypoxia tolerance of *G. petersii* may appear paradoxical for an animal with such an energetically costly brain. *G. petersii* has the ability to regulate the rate of O_2 uptake at low P_{O_2}, displaying a critical value for O_2 consumption at approximately 10 % O_2 saturation. However, the capacity to utilise low ambient levels of O_2 is probably important for protecting the brain from hypoxic damage. Mormyrids inhabit turbid eutrophic tropical waters in which hypoxia is likely to be a common event. In most vertebrates, the brain is strongly dependent on aerobic energy production, being unable to meet its high ATP demand by anaerobic glycolysis alone (Lutz and Nilsson, 1994). In *G. petersii*, the brain is likely to be the first organ to be affected by energy failure during anoxia; the results presented in Table 1 suggest that its brain consumes O_2 at 20 times the rate of the average body tissue. Indeed, bubbling the water with N_2 revealed that *G. petersii* cannot tolerate an ambient O_2 concentration below $0.3\,mg\,l^{-1}$, suggesting that its capacity to produce ATP anaerobically is not sufficient to match its rate of ATP consumption. In nature, it is likely that *G. petersii* relies on gulping air or oxygenated surface water when the ambient O_2 concentration falls below the critical level. Aquatic surface respiration in response to low ambient P_{O_2} is displayed by numerous fish species, especially in the tropics (Kramer and McClure, 1982).

The discharge frequency of the electric organ of *G. petersii* was maintained at a relatively constant rate down to approximately the same critical ambient P_{O_2} as for O_2 consumption (Fig. 2). This indicates that *G. petersii* depresses neither its metabolic rate nor its nervous activity in response to

progressive hypoxia, the discharge rate of the electric organ being under central nervous control (Dye and Meyer, 1986). As in other mormyrid fishes, the electric organ of *G. petersii* delivers electric pulses with a constant amplitude, the electric activity being modulated by changing the discharge rate (Teyssedre and Serrier, 1986). Although not measured in this study, it seems unlikely that this species is able to lower its pulse amplitude in order to save energy in response to progressive hypoxia.

This study reveals that the limit on the proportion of the total energy budget that can be devoted to central nervous activity is much higher than previously known. Since mormyrids seem to be successful fishes, making up some 10 % of the African species of freshwater fish (Roberts, 1975), and are abundant enough to be of local commercial importance (Petr, 1968), it is relevant to ask what advantageous abilities are provided by this exceptionally costly brain. It is likely that these abilities not only include electroreception, a highly effective means of orientation and communication in turbid waters, but also social communication, which appears to be well developed in mormyrids (Kramer, 1990; Moller *et al.* 1989). Interestingly, the weakly electric fish of the group Gymnotoidei of South America, which seem to have a similar electric sensory system (Hopkins, 1983), do not show the extreme increase in brain size displayed by the Mormyridae (Nieuwenhuys and Nicholson, 1969).

Finally, from the present results and from other data discussed above, it is clear that the human brain is not unique in gross physiological terms, neither with regard to its relative or absolute size, nor with regard to its mass-specific or fractional metabolic rate.

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