Physiological Evidence Indicates Lungfish as a Sister Group to the Land Vertebrates

M.L. Glass

Abstract Current research tends to support that lungfish (Dipnoi) and land vertebrates (Tetrapoda) form a sister group, which has stimulated an interest in these animals. The extant lungfish include: *Protopterus,* the African lungfish (four species) and the South American lungfish (*Lepidosiren paradoxa*) (one species). The African and South American lungfish have well-developed lung and reduced gills, while the Australian lungfish (*Neoceratodus forsteri*) is highly dependent on the gill ventilation, and its lung is one of the simplest among vertebrates. Lungfish and land vertebrates share many features of respiratory control. *Lepidosiren* (and probably *Protopterus* possess central cerebral CO_2 and H^+ receptors, which regulate acid–base by increases or decreases in pulmonary ventilation. This regulatory pattern is also valid for land vertebrates, including human beings. By contrast, teleost fish lack central CO_2/H^+-receptors, which suggests that the lung and the central chemoreceptors evolved together. In this context, any very specific features are common to lungfish and land vertebrates, and these include the Hering–Breuer reflex and the presence of very specific stretch receptors.

1 Introduction

Styloichthys, a 417-million-year-old fossil was found, with the characteristics to be expected for a last common ancestor of tetrapods and lungfish (Zhu and Yu 2002). The descendants of the Sarcopterygians (lobe-finned fish) include the coelacanths (Actinistii) with two species *Latimeria chalumnae*, which was discovered in 1938 at the east coast of South Africa, while *L. menadoensis* was seen for the first time

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M.L. Glass
Department of Physiology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Avenida Bandeirantes 3900, 14.049-900 Ribeirão Preto, SP, Brazil,
E-mail: mlglass@rfi.fmrp.usp.br

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in 1999. *Latimeria* possess lungs, but these are filled with fat (Carroll 1988), which makes sense since coelacanths belong to the deep waters. Other descendants of the sarcopterygians include the lungfish (Dipnoi) and the land vertebrates (Tetrapoda), and current research favors the lungfish as the sister group to the tetrapods (Meyer and Dolven 1992; Yokobori et al. 1994; Zardoya et al. 1998; Toyama et al. 2000; Brinkmann et al. 2004). Tetrapod albumin has been studied to a great extent in tetrapods; Metcalf et al. (2007) recently reported its presence in the Australian lungfish (*Neoceratodus forsteri*), and it turned out to have a high degree of similarity to the sequence of tetrapod albumins.

Lepidosireniformes (subclass: Dipnoi; class: Sarcopterygii) include the South American lungfish *Lepidosiren paradoxa*, which the Austrian morphologist Fitzinger (1837) found appropriate to an animal, due to the unexpected combination of a lung and a gill system. This slender lungfish is equipped with appendages and can weigh about 1 kg (Fig. 1), and its popular name is ‘pirambóia’, taken from the language of the Tupi indians. *L. paradoxa* is found within the Amazon and Paraná-Paraguai regions and inhabits shallow vegetation-covered lakes. Mainly, *L. paradoxa* feeds on invertebrates, including mollusks that can easily be crushed by its tooth plates (Sawaya 1946).

The African lungfish *Protopterus* includes four species (*amphibius*, *annectens*, *aethiopicus* and *dollei*). These lungfish are similar to *L. paradoxa,* although more heavily built, and their habitats are much like those described above. *L. paradoxa* and *Protopterus sp.* are crucially dependent on the lung for gas exchange, while the Australian lungfish (*Neoceratodus forsteri*) has a well-developed gill system

Fig. 1 Photo of the South American lungfish (*Lepidosiren paradoxa*) in the laboratory, making a slow but elegant turn

combined with a simple lung, which is ventilated at very long intervals (one h or more) (Kind et al. 2002). It inhabits slowly moving rivers of the Queensland region of Australia, where it may reach 1.5 m and a weight of 40 kg.

In the nineteenth and twentieth centuries, much effort was devoted to comparative morphology and anatomy, which was backed by the concepts of evolutionary biology and paleontology. Recently, techniques based on amino acid sequences and mitochondrial genome DNA sequences have provided criteria to evaluate the most probable cladograms for specific groups of animals (cf Yokibori et al. 1994; Brinkmann et al. 2004). Clearly, comparative physiology can be used to trace back the origins and evolution of physiological mechanisms. In this chapter, we will focus on some mechanisms of ancient origin. One example is the dual locations of CO_2/H^+-receptors: the peripheral chemoreceptors monitor acid–base status of the blood, while the central chemoreceptors are stimulated by changes of acid–base status of the CSF and the interstitial environment. This would have pleased the Greek philosopher Anaximander, who about 2,500 years ago stated that once man was another animal, perhaps a fish.

2 How Advanced is the Lungfish Lung?

Surfactant is a substance that reduces surface tension of the lung, and it contains disaturated and unsaturated phospholipids along with surfactant proteins. A lung cannot function without surfactant and, interestingly, surfactant is also present in swim bladders and gill systems, and is produced by type II cells (Daniels et al. 2003); see also Chap. . Lepidosireniformes (*Protopterus* and *Lepidosiren*) produce surfactants that are very similar to those of amphibians, whereas *Neoceratodus* has a lipid composition, which is more closely related to that of actinopterygian fish (Orgeig and Daniels 1995). In the same context, Power et al. (1999) stated that the surfactant composition of *Neoceratodus* has been preserved over the last 300 million years. Only the right lung develops in *N. forsteri*, and it receives blood from both pulmonary arteries. Further, the lung is in a dorsal position, while lepidosireniformes and land vertebrates possess bilaterally positioned lungs in a ventral position (Perry 2007). Bassi et al. (2005) reported pulmonary diffusing capacity in *L. paradoxa*, using the equation: $\mathrm{D_LO_2} = \dot{V}\mathrm{O_2} \cdot \Delta\mathrm{PO_2^{-1}}$ (Bohr 1909), in which the individual components are: $D_\mathrm{L}\mathrm{O_2}$ = diffusing capacity; $\dot{V}\mathrm{O_2} = \mathrm{O_2}$ flux through the tissue membrane, which separates lung gas and pulmonary capillary blood, or in general O_2 uptake; $\Delta\mathrm{PO_2}$ = the O_2 pressure gradient between lung gas and pulmonary capillary blood. $D_\mathrm{L}\mathrm{O_2}$ was 0.044 mLSTPD $\mathrm{kg^{-1}\,min^{-1}}$ at 35°C (Bassi et al. 2005), which is close to the value for a bullfrog (*Rana catesbeiana*), which has a $D_L\mathrm{O_2}$ of 0.054 mLSTPD $\mathrm{kg^{-1}\,min^{-1}}$ (Glass et al. 1981a). Further, Crawford et al. (1976) report the rather high $D_\mathrm{L}\mathrm{CO}$ of 0.068 mLSTPD $\cdot\,\mathrm{kg^{-1}\,min^{-1}}$ for the Greek turtle (*Testudo greca*).

The lung of *L. paradoxa* was studied by morphometric measurements, which provided the amazing information that 99% of its gas exchange surface belongs to the lung, while the skin and the rudimentary gills account for the remaining 1% (Moraes et al. 2005). On the other hand, Sanchez et al. (2005) reported that aquatic O_2 uptake accounted for 9% of total uptake. This seeming contradiction can be solved, because Moraes et al. (2005) found a high density of capillaries in all parts of the gill system, and a respiratory role of the gills cannot be excluded.

The D_LO_2-values for *L. paradoxa* and a bullfrog are very similar, whereas the diffusing capacity of the tegu (*Tupinambis sp.*) and the monitor lizards (*Varanus exanthematicus*) is twofold greater than *L. paradoxa* (Glass and Johansen 1982; Glass et al. 1981a, b). The transition from ectothermic to endothermic metabolism greatly increased metabolism which is not surprising, considering that an alveolar mammalian lung has a 16-fold higher $\mathrm{D_LO_2}$ than *L. paradoxa* (Takezawa et al. 1980).

3 Regulation of Acid–Base Status and Oxygen Levels

As explained in Chap. 3, true lungs are found in land vertebrates, lungfish and bichirs (Subclass: Actinopterygii — ray-finned fish. Order: Polypteriformes). Currently, information on respiratory control in land vertebrates is increasing rapidly, and the interest in lungfish is growing. By contrast, the information on the respiratory physiology of bichirs is scarce (see Chap. 3). Holeost and teleost fish regulate acid–base status by ion exchange (Heisler 1984), because O_2 homeostasis has a priority due to the ever-changing O_2 levels of the aquatic environment (Dejours 1981). Oxygen receptors are located within the gill system, where receptor groups screen the blood or the inspired water (Soncini and Glass 2000; Burleson and Milsom 1995a,b). Acid–base regulation of teleost fish depends on cells located in the gill epithelia, and accounts for no less than 90% of the acid–base relevant ion transfers, while the kidney contributes the remaining 10% (Heisler 1984; Claiborne and Heisler 1986).

In land vertebrates and lungfish, the ability to regulate acid–base status and O_2 homeostasis depends on adjustments of the ratio

$$V_{EF}/V\mathrm{CO_2} = \mathrm{RT}/P_{EF}\mathrm{CO_2},$$

where V_{EF} = effective ventilation of the lung, $V\mathrm{CO_2}$ = pulmonary $\mathrm{CO_2}$ output, R = the gas constant, and T = absolute temperature (°K), and the equation is derived from the general gas law. In mammalian respiratory physiology, the equation is usually referred to as “the alveolar ventilation equation”. Mammals are the only land vertebrates equipped with alveolar lungs, but ‘alveolar’ ventilation can be substituted by ‘effective ventilation’ of a lung.

Land vertebrates (Tetrapoda) control acid–base status by means of central and peripheral $\mathrm{CO_2/H^+}$-receptors, and by far most information is available for mammals. The passage of $\mathrm{H^+}$ and $\mathrm{HCO_3^-}$ through the blood–brain barrier is very limited, whereas $\mathrm{CO_2}$ traverses. Therefore, in a classical study Loeschcke et al. (1958)

assumed that CO_2 would react with water according to the equation: $CO_2 + H_2O \Leftrightarrow H^+ + HCO_3^-$, which would leave H^+ as the stimulus. As a further progress, H^+ receptors were detected in a bilateral position within the ventral part of the medulla oblongata (Schläfke et al. 1975). We now know that more sites are involved, including the retrotrapezoid nucleus (Guyenet et al. 2005) and the midline raphe (Bernard et al. 1996). The peripheral chemoreceptors are of very ancient origin, and the chemoreceptors of the aortic arch of reptiles and birds and the carotid bifurcation of mammals and amphibians are homologous to the O_2-receptors on the first gill arch of fish (Milsom 2002). It is, therefore, not surprising that the peripheral receptors include the O_2-sensitive glomus cells (For further information see Nattie (1999; 2006)). Studying one specimen of *Protopterus*, Lahiri et al. (1970) injected the afferent gill arteries with hypoxic blood and cyanide, which increased ventilatory movements. As further evidence, bilateral section of the first three gill arches reduced responses to the stimuli, but the procedure would not define the exact locations of the O_2-sensitive sites.

4 Respiratory Control in Lungfish Compared to Amphibians and Other Land Vertebrates

Striedter (2005) stated: "Lungfish brains exhibit very little histological differentiation, and are among the simplest vertebrate brains". This is true; but in some regards, they possess characteristics which strikingly resemble those of the land vertebrates. The Lepidosireniformes (*Protopterus* and *Lepidosiren*) have amazingly high $PaCO_2$, high bicarbonate and a low pHa. As an example, at 35°C PaO_2 was 76 mmHg, $PaCO_2$ 30 mmHg, pHa 7.39 and plasma $[HCO_3^-]$ 25.9 mM (Bassi et al. 2005). The corresponding values at 25°C were PaO_2 81 mmHg, $PaCO_2$ 21 mmHg, pHa 7.53, and plasma $[HCO_3^-]$ 20.0 mM. The values for *Protopterus dolloi* have the same tendency: PaO_2 66 mmHg, $PaCO_2$ 18 mmHg, pHa 7.37 (Perry et al. 2007). This is very distinct from the values for anuran amphibians such as the cane toad (*Chaunus schneideri*), with PaO_2 61 mmHg, $PaCO_2$ 7.7 mmHg, pHa 7.75, and plasma $[HCO_3^-]$ 13.7 mM (Wang et al. 1998). *Neoceratodus* (Ceratodontidae) is certainly different from the lepidosireniformes. Its values are PaO_2 39 mmHg, $PaCO_2$ 4 mmHg, pHa 7.64 (Lenfant et al. 1966, 1967), which reflects its predominant gill respiration and Dejours (1981) has pointed out that the more an animal depends on aquatic respiration, the lower its $PaCO_2$. It should be noted that some early studies report very low PaO_2 values, which are unrealistic due to invasive techniques and/or incorrect handling of the blood samples.

5 Focus on the South American Lungfish *L. paradoxa*

Figure 1 shows the eel-like body of *L. paradoxa*, with which Johansen and Lenfant (1967) obtained pioneering data, in particular concerning gill function. They measured an O_2 extraction from the gills (EO_2) of 30%, which is low compared to

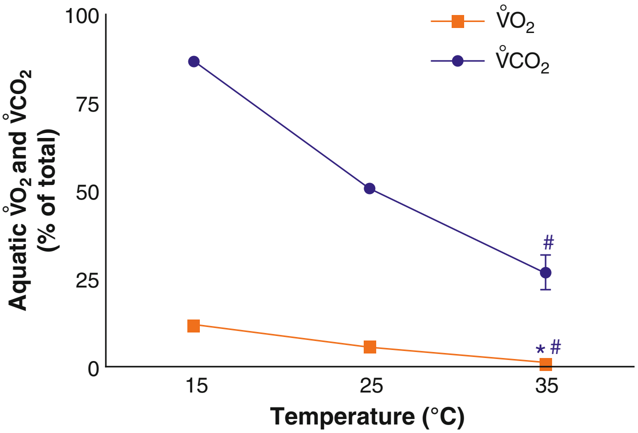

Fig. 2 *Upper curve*: Carbon dioxide elimination to the water as a function of total CO_2 output. *Lower curve*: Total O_2 uptake from the water. The temperature range is 15–35°C. Increasing temperatures reduced the percentage CO_2 output to the water, because a larger percentage of total CO_2 became redirected and eliminated by pulmonary ventilation. A small amount of O_2 was taken up from the water at 15°C, whereas the uptake was practically nil at 35°C, which confirms a high dependence of *L. paradoxa* on lung ventilation (ANOVA, log-nat. transform., Bonferroni, Friedman, Dunn. Mean ± SEM, $n = 5$)

teleost fish, which reach no less than 70–85% (Rantin et al. 2007). Further, they discovered that the gill arches 1 and 2 are practically devoid of gill filaments, and the remaining arches had considerably reduced surface areas. A high density of capillaries might, however, account for some O_2 uptake by the gills, since the proper respiratory exchange surface is negligible (Moraes et al. 2005).

The relative roles of aquatic and aerial gas exchanges were assessed by Amin-Naves et al. (2004), who focused on a temperature range from 15 to 35°C. Aquatic O_2 uptake by the animal was minute, and constant with temperature (about 0.01 mlSTPD kg^{-1} min^{-1}), while pulmonary O_2 uptake increased from 0.06 (15°C) to 0.73 (35°C) mlSTPD kg^{-1} min^{-1}, while pulmonary ventilation increased 17-fold over the same temperature range. Figure 2 shows the pulmonary and aquatic gas exchanges, presented as percentage values for $\dot{V}O_2$ and $\dot{V}CO_2$ at the three tested temperatures. It is clear that the aquatic $\dot{V}O_2$ is practically nil at 35°C, while the lung has taken over. Concurrently, aquatic CO_2 elimination strongly dominates at 15°C, but becomes reduced as temperature increases, and at 35°C only 1/3 of the total CO_2 output is eliminated to the water, while the lung eliminates 2/3 of the remaining output. This occurs because the higher the temperature, the more dominant becomes the gas exchange by the lung.

L. paradoxa possess central chemoreceptors, which were first detected using superfusion of the 4th cerebral ventricle with mock CSF solutions at pH levels ranging from 7.4 to 8.0, while pulmonary ventilation was measured using a method for freely diving animals. A reduction of pH from 8.0 to 7.4 increased ventilation threefold, while respiratory frequency increased from 5 to 12 breaths h^{-1} (Sanchez et al. 2001a). The next step was to verify the hypothesis that the *L. paradoxa* possess peripheral CO_2/H^+-receptors. To test this, the lungfish was initially kept in aerated water, after which combined aquatic/gas-phase hypercarbia ($PCO_2 = 49\,mmHg$) was maintained for 5 h, during which pulmonary ventilation gradually increased 8-fold relative to the initial control value. In a second run, this procedure was repeated with the modification that superfusion of normocarbic mock CSF was applied during the last 2 h of the experiment. This reduced ventilation which, however, remained 3-fold higher than the initial control value. In addition, this hyperventilation was statistically different both from the control value and from the maximum response. With this information, it could be calculated that peripheral CO_2/H^+-receptors accounted for 20% of the ventilatory drive, whereas the bulk part of the drive was central (Amin-Naves et al. 2007a,b).

This was consistent with data on the central chemoreceptor drive in the cane toad *Chaunus schneideri*, and in the alligator (Branco and Wood 1993). The value for mammals is from Smith et al. (2006), and the measurement for birds (duck) is from Milsom et al. (1981). See Fig. 3 for a cladogram that informs on peripheral and central components to the CO_2/H^+ receptor drive in various groups of vertebrates.

Shams (1985) exposed the medulla of anaesthetized cats to an increased PCO_2 while pH was kept constant by superfusion, which stimulated pulmonary ventilation. Harada et al. (1985) studied the brainstem of the newborn rat, and found that hypercarbia increased the respiratory output of the phrenic nerve, while pH was kept constant. Toads (*Chaunus schneideri*. Previously *Bufo paracnemis*) were also evaluated in this context, and it turned out that an increase of mock CSF CO_2 increased ventilation, while pH was kept constant.

These two stimuli (CO_2 and pH) were also tested in *L. paradoxa* and the acid–base environment of the central chemoreceptors was controlled by superfusion, while pulmonary ventilation was recorded. Initially, superfusion was applied to keep $PaCO_2$ at 21 mmHg and pH at 7.45, which corresponds to normal values for animals in the water at 25°C. As a second step, pH continued at 7.45, while PCO_2 was increased to 42 mmHg, and this increased pulmonary ventilation twofold. Conversely, ventilation increased 3-fold, when mock CSF pH was reduced from 7.45 to 7.20, while PCO_2 was kept constant at 21 mmHg.

Peripheral chemoreceptors in mammals also respond to both CO_2 and H^+(cf. Hlastala and Berger 1996), and the advantage of this dual mode of stimulation might be that ventilation can respond to both respiratory and metabolic acidosis.

Evidently, lungfish and land vertebrates share characteristics of respiratory regulation, and the origins of key elements are clearly very ancient. A large number of non-mammalian land vertebrates are equipped with intrapulmonary stretch receptors, in which the firing rate becomes reduced by increases of CO_2 (cf. Milsom et al. 2004). Slowly adapting stretch receptors were discovered in *Protopterus* and *L. paradoxa* (DeLaney et al. 1983), and it turned that increased intrapulmonary CO_2 levels inhibited the firing rate of the receptors. Rapidly adapting receptors were also found, but the slowly adapting type was more common. The firing rate of the slowly adapting receptors was dependent both on rate of inflation and on CO_2 levels. Curiously, such types of receptors have been found in the air-breathing organ of the

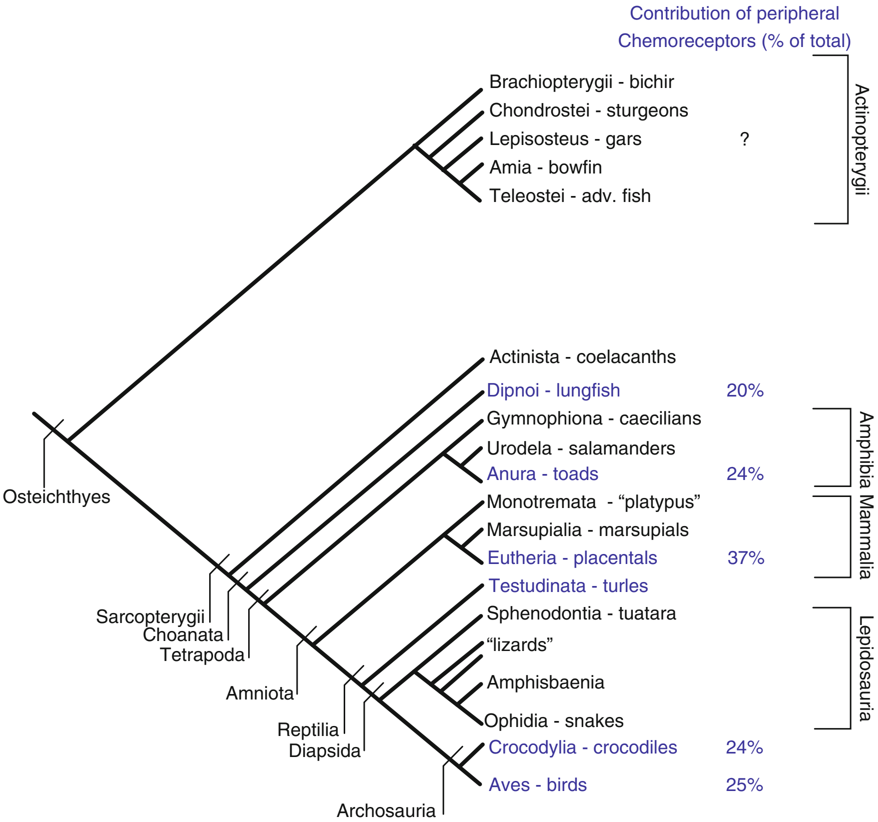

Fig. 3 Cladogram representing probable relationships between the vertebrate groups. *Blue* indicates that the peripheral contribution to the peripheral chemoreceptor drive is known for at least one species of the group. The references are: (1) dipnoi: lungfish *L. paradoxa* 20% (Bassi et al. 2005), (2) anura: toad *Chaunus schneideri* (earlier *Bufo paracnemis*) (Branco et al. 1993), (3) eutheria (placentals): dog 37% (Smith et al. 2006), (4) crocodylia — crocodiles 24%, Branco and Wood (1993), (5) aves — pekin duck 25% (Milsom et al. 1981; Shams and Scheid 1989)

gar *L. oculatus* (Smatresk and Azizi 1987). Two types of receptors were identified. Like in the lungfish, a rapidly adapting receptor was present, and a slowly adapting type was CO_2-sensitive. Further, hypercarbia reduced the firing rate of the slowly adapting receptors, which might suggest an ancient origin rather than a coincidence.

It is easy to detect CO_2-sensitive stretch receptors. Initially the animal breathes air, after which hypercarbia is applied, which increases pulmonary ventilation at a fixed CO_2 level. After some time, the animal suddenly returns to air-breathing. At this point, one would expect a decrease of ventilation. Instead, ventilation increases steeply, because the intrapulmonary CO_2 levels become reduced, which removes the inhibitory action of the CO_2-sensitive stretch receptors. This effect is often referred to as a 'post-hypercapnic hyperpnea' (Milsom et al. 2004). *L. paradoxa*

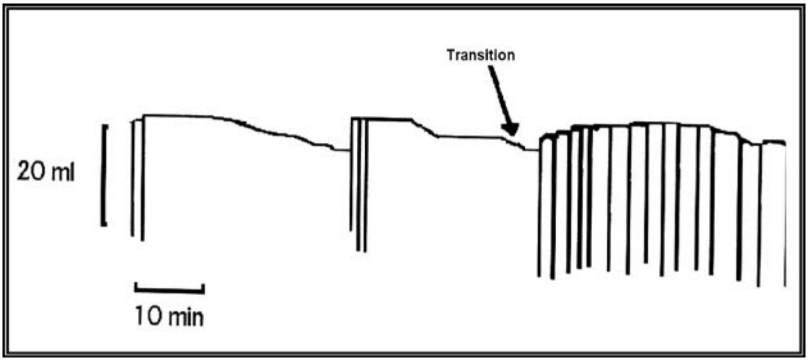

Fig. 4 This figure illustrates the presence of 'post-hypercapnic hyperpnea' in *L. paradoxa.* The recording shows the tidal volume during steady state hypercarbia. Both gas phase and water were kept at $PCO_2 = 55\,mmHg$, after the inspired gas was substituted with air. This reduced intrapulmonary CO_2 levels, which stimulated ventilation and caused a transient burst of intensive respiration. This response is a hallmark for the present of intrapulmonary CO_2-sensitive stretch receptors

was tested in that regard, and the recording shows the expected response (Sanchez and Glass 2001); see Fig. 4.

O_2-receptor function has also been studied in *L. paradoxa*, including an evaluation of ventilatory responses to aerial and/or aquatic hypoxia. It turned out that aquatic hypoxia (range 145–153 mmHg; $t = 25°C$) had no effect on pulmonary ventilation, whereas gas phase hypoxia caused a fourfold increase of ventilation. In addition, the O_2 stimulus (O_2 content or, alternatively, O_2 partial pressure) was identified, since a reduction of O_2 content by 50% had no effect on ventilation. This proves that the specific O_2 stimulus is O_2 partial pressure and not O_2 content (Sanchez et al. 2001b). Amphibians such as *Chaunus schneideri* also possess O_2 receptors that monitor O_2 partial pressure, and this modality seems to apply to most land vertebrates, including human beings (Wang et al. 1994; Branco and Glass 1995).

6 Focus on the African Lungfish *Protopterus sp*

Smith 1935) initiated a line of studies on kidney physiology, including *P. aethiopicus* (Smith 1930). In an early pioneering work, Johansen and Lenfant 1968) studied gill function and the relative importance of gas exchange surfaces at 20°C. In this species, the O_2 extraction by the gills ranged from 11 to 36%, which is very low considering that the normal value for teleost fish is about 85% (Rantin et al. 2007), but this is consistent with data for *L. paradoxa*. In addition, Johansen and Lenfant (1968) reported that the O_2 uptale from the water accounted for as little as 11% of total uptake. By contrast, the CO_2 output to the water accounted for no less than 73% of total CO_2 output. The corresponding values for *L. paradoxa* at 25°C were an aquatic O_2-uptake of 10% of total uptake, combined with 65% of CO_2 produced eliminated to the water (Amin-Naves et al. 2004). The values of *Protopterus sp.* and *L. paradoxa* are strikingly similar, which is surprising since the split of South America from Africa took place some 100 million years ago (Vidal et al. 2007).

Recently, Perry et al. (2007) studied acid–base regulation in the *P. annectens* assuming that ventilatory responses to hypercarbia would occur which, in turn, would indicate the presence of chemoreceptors. A 1-h acid infusion was applied, which temporarily increased respiratory frequency of the gills and lung twofold, after which these values dropped to reach the previous baseline within 5 h. The procedure can not distinguish between central and peripheral chemoreceptors. Nevertheless, the respiratory responses of *P. annectans* were large and immediate, and clearly distinct from the weak responses of teleost fish to acid–base disturbances (Soncini and Glass 2000). The authors also found that extra-renal routes were a key factor in metabolic compensation. On the other hand, during aestivation in *P. aethiopicus*, plasma $[HCO_3^-]$ gradually increased over 7 months, but the mechanism of the increase was not clear, and a possible explanation could be a gradual loss of body water, concentrating plasma $[HCO_3^-]$ (DeLaney et al. 1977).

Compensation of pHa was not evident in *L. paradoxa*, in spite of an exposure to 7% CO_2 (49 mmHg) during 48 h (Sanchez et al. 2005). It should be pointed out that its gills are considerably more reduced than those of *Protopterus sp.*. As a possibility, the pH regulation could be similar to that of salamanders studied by Heisler et al. (1982). Urodeles inhabit a strenuous hypercarbic environment, which makes the regulation of the extracellular environment difficult, whereas the intracellular compartments are regulated in response to hypercarbia.

Carbonic anhydrase (CA) has one of the fastest turnover numbers of all enzymes. Nevertheless, recent studies report that addition of CA will increase CO_2 excretion (Gilmour et al. 2007). Thus, bovine CA slightly decreased $PaCO_2$ of *P. dolloi*, while pH increased from 7.48 to 7.53. The authors conclude that the bulk parts of O_2 and CO_2-excretion occurs by the lung, which is consistent with data on *L. paradoxa* exposed to temperatures from 15 to 35°C (Amin-Naves et al. 2004).

Both amphibians and lungfish inflate the lung by positive pressure, using the buccal cavity as a force pump that inflates the lung with the inspired gas (McMahon 1969). Lungfish have ribs, but these are not activated during respiration (Foxon and Bishop 1968). Reaching the surface, *Protopterus* closes the mouth and compresses the buccal cavity to eject water through the operculum. As the second step, the opercular and buccal spaces expand, while air enters by the mouth. Subsequently, a glottal sphincter opens and the gas is expelled from the lung. After that, several stepwise movements force the gas into the lung (McMahon 1969; Lomholt 1993). These rather complicated movements invite the question asked by Pack et al. (1992) about a possible action of a Hering–Breuer reflex in response to the lung inflation. To this end, the authors placed a tube into the lung of the animal, which allowed to more volume to be added at the onset of the buccal force pump, which would shorten the time for inspiratory buccal force movements. In addition, it turned out that vagotomy would virtually abolish the relationship between inflation of the lung and expansion of the lung. The presence of this mechanism in a lungfish indicates that a control of lung expansion is a basal feature. See also Pack et al. (1990).

7 Focus on the Australian Lungfish *Neoceratodus Forsteri*

The Australian lungfish, *Neoceratodus forsteri* Krefft, is heavily built and reminds us of ancestors such as the Devonian *Dipterus* (Carroll 1988). As a new and surprising development, this lungfish turns out to be an obligate neotene animal, or in other words, it can be considered as a larval form with capacity to produce, which is backed up by deficiencies in its thyroid function. Concurrently, possible neotenic features in *Lepidosiren* and *Protopterus* are under discussion (Joss and Johanson 2007). *N. forsteri* inhabits river systems in the South-East Queensland region, and aestivation has never been reported for this lungfish. Surprisingly, *N. forsteri* possess ampullary organs that may be used to locate the prey. Based on application of various stimuli, the authors confirmed that *N. forsteri* can detect weak electric fields surrounding living animals, and they also propose that the fish uses this information to locate hidden prey (Watt et al. 1999). To my knowledge, there is no similar information for *Lepidosiren* and *Protopterus*.

Aestivation is absent in *N. forsteri*, which makes sense, since this animal is not exposed to the strenuous environmental conditions of Lepidosireniformes. Johansen et al. (1967) studied respiratory function in *N. forsteri*, and found that the interval of air-breaths often lasted more than 1 h or more (temp. 18°C). This is not surprising, because its gill system is highly developed, whereas the lung is very simple, when compared to those of other extant lungfish. As could be expected, hypoxia provoked large increases of branchial and pulmonary ventilation. Later, Kind et al. (2002) reported a nearly 8-fold increase of air-breaths, with reduction of O_2 from 120 mmHg to 40 mmHg. Air-breathing was always accompanied by a burst of branchial movements and a large increase of pulmonary perfusion (Fritsche et al. 1993). Based on the principle of Dejours 1981), a high dependence on gill respiration leads to a low $PaCO_2$, and the blood gas values ($PCO_2 = 3.6$ mmHg and $pHa = 7.64$; $t = 18°C$) are close to those for teleost fish. By contrast, *L. paradoxa* and *Protopterus sp*. are highly dependent on the lung, which is reflected in their high $PaCO_2$ values and low pHa.

8 Aestivation

Aestivation is a seasonal dormancy, which is usually related to adverse environmental conditions such as a dry season and/or limited availability of food items. Different from hibernation, aestivation can occur without any temperature changes.

Aestivation is well-studied in amphibians, and information on reptiles is growing (Abe 1995; Andrade et al. 2004). In amphibians, the reductions in O_2 uptake ranged from 18 to 54% relative to previous baseline value for awake, undisturbed animals (Glass et al. 1997).

DeLaney et al. (1974) decided to address aestivation in *P. aethiopicus.* Initially the lungfish caved out a burrow, when the mud gradually dried out, and this was followed by mucous secretion from the skin of the animal. Soon, the mucous hardened to cover the animal, except for an opening at the mouth, which allowed respiration via a breathing channel. The transition from water to cocooned conditions, correlated with reduction of O_2 uptake to half of the previous value for the animal in water (DeLaney et al. 1974). Over 2 weeks the mean blood pressure fell from 24 mmHg to 15 mmHg, and f_H decreased from 35 to 11–16 beats min^{-1}. As an extreme case, Lomholt (1993) reported that *P. amphibius* could remain in the cocooned state for 6–7 years, and in one specimen the O_2 uptake had decreased to 15% of the initial value for the animal in water. Using X-rays, Lomholt (1993) claimed that one of the animals (0.4 kg) occupied the entire space of the burrow, which would require a lung volume of 250 ml. The survival time for cocooned *Protopterus* is amazing, but one *P. amphibius* achieved a possible record, with 7 years of survival (Lomholt 1993). If the animal really fills out the space of the burrow, then it should be possible to apply pneutachography, since inspiration and expiration would be the only major movements. Later, DeLaney et al. (1977) studied the effects of aestivation on blood gases of *P. aethiopicus* at 25°C. With the animal in water the values were: $\mathrm{PaCO_2} = 26\,\mathrm{mmHg}$; $\mathrm{pHa} = 7.60$. During the second weak of aestivation, $\mathrm{PaCO_2}$ increased at a lower rate to reach a $\mathrm{PaCO_2}$ of 49.8 mmHg, with a pHa of 7.37. These changes can be explained based on several mechanisms. When in water, a large fraction of the CO_2 output becomes eliminated to the water. Assuming that the observation of a close fit to the burrow is correct (Lomholt 1993), then the whole CO_2 output would be eliminated by the lung. Once again the principle of Dejours (1981) can be applied to predict a large increase of $\mathrm{PaCO_2}$ which, consequently, lowers pHa. The authors were, however, uncertain about the nature of a slow increase of plasma $[\mathrm{HCO_3^-}]$. DeLaney et al. (1974) reported a downregulation of mean blood pressure, and a heart rate that was reduced to 50% of the value for the animal in water. The breathing frequency decreased from 20 to 8 breath h^{-1} (values before and after aestivation). Unfortunately, respiratory signals provide no informationregarding the possible reduction of V_T after transition to aestivation. Returning to long-term data, one *P. amphibius* that had been in the cocoon for 6 years had an end-tidal $\mathrm{PCO_2}$ of 40 mmHg and an end-tidal $\mathrm{PO_2}$ of 120 mmHg, which would practically fit into the data for some weeks of aestivation (Lomholt 1993).

Recently, Perry et al. (2008) studied aestivation in *P. dolloi*, and under favorable conditions in the laboratory would induce secretion of a cocoon, which became hard after 4–5 days. Before aestivation, *P. dolloi* consumed $0.35\,\mathrm{mlSTPD\,kg^{-1}\,min^{-1}}$ (5°C) but in the cocoon the O_2 uptake increased to $0.45\,\mathrm{mlSTPD\,kg^{-1}\,min^{-1}}$, which reduced $\mathrm{PaCO_2}$ from 18 to 14 mmHg. Greenwood 1986) states, however, that this lungfish does not aestivate in its normal habitat. The authors realized that the animals were not in a state of aestivation and, therefore, coined the word ‘terrestrialization’,

Table 1 Blood gases in *Lepidosiren* in water and after 40 days of aestivation

	In water	*Aestivation 40 days*
PaO_2 (mmHg)	87.7 ± 2	$77.1 \pm 3^*$
$PaCO_2$(mmHg)	21.8 ± 0.4	$34.4 \pm 3.2^*$
pHa	7.51 ± 0.05	7.53 ± 0.05

Mean values ± SEM; $n = 5$ ($p < 0.05$ paired t-test)

and this condition was maintained for more than 1 month, which is important, because true aestivation could have appeared after a longer period. The solution to this enigma would be highly interesting, and interactions between various disciplines would be required.

The South American lungfish also aestivates, but for much shorter periods, typically 1 or 2 months. When a lake dries out, the aestivation is initiated by a position in which it assumes a U-shape in which the tail approaches the head. For breathing, the animal slides the head upward, and breaks the surface to respire. There is no cocoon formation, but the characteristics of aestivation are very similar as seen from Table 1.

In conclusion, it is clear that lungfish and land vertebrates share very fundamental mechanisms of physiological regulation. These concern the control of pulmonary ventilation. These involve the relative drives of central and peripheral CO_2/H^+ receptors, where their relative roles are very similar. Moreover, in mammals, toads and lungfish the common central stimuli are both CO_2 and H^+, and intrapulmonary stretch receptors that lower firing rate if intrapulmonary PCO_2 increases are also found in lungfish as well as in tetrapods. A Hering–Breuer reflex is even present in lungfish, which once again shows common traits which most likely evolved before the now rather likely ramification between lungfish and tetrapods.

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