

THE EFFECTS OF HYPERCAPNIA ON THE ARTERIAL ACID-BASE STATUS IN THE TEGU LIZARD, *TUPINAMBIS NIGROPUNCTATUS*

BY M. L. GLASS* AND N. HEISLER

*Abteilung Physiologie, Max-Planck-Institut für experimentelle Medizin,
D-3400 Göttingen, FRG*

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SUMMARY

The effects of hypercapnia on the arterial acid-base status of the Tegu lizard, *Tupinambis nigropunctatus* (Spix), were studied at 25°C. Arterial P_{CO_2} increased over the first 2 h of hypercapnia causing a fall in arterial plasma pH (pH_a) of about 0.17 units with $\approx 4\%$ CO_2 and about 0.3 units with $\approx 7\%$ CO_2 . In both conditions, plasma pH increased slightly (≈ 0.02 units) between 2 and 72 h. Plasma $[\text{HCO}_3^-]$ rose during the initial increase of Pa_{CO_2} (by approx. 5.5 mmol l^{-1} with $\approx 7\%$ CO_2 ; approx. 1.9 mmol l^{-1} with $\approx 4\%$ CO_2) during the first 2 h, and further increased by 4 mmol l^{-1} between 2 and 72 h of hypercapnia, while Pa_{CO_2} did not change. The increases of plasma $[\text{HCO}_3^-]$ resulted in a recovery of pH_a by 38 or 32% (≈ 4 and $\approx 7\%$ CO_2 , respectively) relative to the fall of pH_a that would occur at constant $[\text{HCO}_3^-]$.

The limited and incomplete compensation of pH_a during environmental hypercapnia is consistent with data for other air-breathing ectothermic vertebrates, and contrasts with the typical response of water-breathing fish, in which compensation is usually complete.

INTRODUCTION

Several studies have reported that reptiles may regulate their arterial acid-base status by adjustment of P_{CO_2} via changes in pulmonary ventilation in response to hypercapnia (Wood & Lenfant, 1976; Jackson, 1978; Glass & Wood, 1983). In contrast, the information on adjustments of arterial pH by changes in plasma bicarbonate concentration ($[\text{HCO}_3^-]$) is scarce in reptiles. This lack of information is surprising, because such data are available for several other groups of ectothermic vertebrates. In water-breathing fish, increased extracellular $[\text{HCO}_3^-]$ may almost offset the effects of hypercapnia on arterial pH (Heisler, Weitz & Weitz, 1976; Eddy, Lomholt, Weber & Johansen, 1977; Toews, Holeton & Heisler, 1983;

*Present address: Department of Zoophysiology, University of Aarhus, DK-8000 Aarhus, Denmark.

Heisler, 1984). In contrast, amphibians are incapable of large increases in extracellular $[\text{HCO}_3^-]$, and this limits pH restoration during hypercapnia (Toews & Heisler, 1982; Heisler, Forcht, Ultsch & Anderson, 1982).

The present study provides data for the effects of hypercapnia on the arterial acid–base status of a reptile, the Tegu lizard, *Tupinambis nigropunctatus*. It was focused on the time courses of the changes in plasma pH, P_{CO_2} and $[\text{HCO}_3^-]$ during exposure to elevated environmental P_{CO_2} , and the capability of a reptile to compensate hypercapnia-induced acid–base disturbances by elevations of plasma bicarbonate concentration.

MATERIALS AND METHODS

Tegu lizards (*Tupinambis nigropunctatus*) were purchased from a commercial animal supplier. They were kept in large glass terraria for several months before experimentation at temperatures between 35 and 20°C in a day/night cycle of about 12/12 h and the facility to regulate body temperature by behavioural means under infrared radiators. They were fed on mice, rats, beef liver and meat. Experiments were performed on 15 specimens with body weights ranging from 0.81 to 1.33 kg (mean 1.10 kg).

Arterial cannulation

Anaesthesia of the lizards was initiated by exposure to halothane vapour in a closed glass aquarium (Heisler, Neumann & Maloiy, 1983). After the animals had lost reactivity, a tube was inserted into the trachea *via* the mouth and glottis, and was connected to a small animal respirator equipped with a halothane evaporator. During surgery the lizard was ventilated at normal rates with air containing $\approx 1\%$ halothane.

The left carotid artery was occlusively cannulated with PE 50 or PE 60 tubing *via* a lateral neck incision (2–3 cm). The catheter was fed out through the skin at a point in front of the skin incision close to the ear of the animal. The incisions were carefully closed by atraumatic sutures and the catheter was firmly fixed to the skin of the neck.

After cannulation a dose of chloramphenicol was administered (20 mg kg^{-1} ; i.m.) and the catheters were flushed with heparinized reptile Ringer solution (200 i.u. ml^{-1} ; de la Lande, Tyler & Pridmore, 1962) and subsequently flushed again every 12 h. During the recovery period the lizard was artificially ventilated with air until spontaneous breathing efforts were observed (usually after 0.5–1 h). The experiments were initiated 24–48 h after recovery from surgery, at which time the blood acid–base status had returned to control values for at least 20 h. The animal was supplied with drinking water *ad libitum* during recovery as well as during the experiments, whereas food was withheld.

After the experiment, the lizard was again anaesthetized, the catheter was removed and the artery tied off. The skin incision was closed and the lizard treated again with antibiotics. The incision healed without complications, the animals recovered quickly and were kept after this treatment in some cases for much longer than a year.

Protocol

The lizard was enclosed in a Plexiglas cylinder (about 20 cm i.d., 60 cm long), but could move freely within the tube. The cylinder was thermostatted at 25°C and shielded against visual disturbances. During experiments the 12/12 h light/dark cycles were continued and the Plexiglas cylinder flushed with air or hypercapnic gas mixtures (about 4 or 7% CO₂ with 20% O₂ and balance N₂) delivered from a Wösthoff gas mixing pump at about 600 ml min⁻¹ (Wösthoff GmbH, Bochum, FRG).

After transfer of the animals into the experimental set-up (which was performed at 07.30 h, when the *Tegus* were inactive and relaxed), the experiment was initiated by a 6–26 h control period, during which the arterial pH (pH_a) was determined repeatedly to assure steady-state conditions. At the end of this period, one or two blood samples were analysed for control pH, P_{CO₂}, P_{O₂} and [HCO₃⁻]. Then the gas supply to the animal container was switched to one of the hypercapnic gas mixtures (≈4 or ≈7% CO₂) and blood was sampled and analysed for the above parameters at time 0.5, 1, 2, 4, 6, 8, 24, 48 and 72 h after initiation of hypercapnia. Each animal was exposed to only one of the hypercapnic gas mixtures because the arterial catheters were not patent for a sufficiently long time period.

In order to test the possibility that the bicarbonate accumulation in the extracellular space was limited by the renal H⁺ excretion rate, about 5 mmol kg⁻¹ body weight of bicarbonate was infused intra-arterially over a time period of 1 h into three specimens, after they had been exposed to ≈7% CO₂ for 24 h with continuing hypercapnia, and the acid–base status was monitored during the next 12 h.

Blood analysis for pH, P_{CO₂} and P_{O₂} was performed by means of electrodes thermostatted to 25°C (BMS 3, Radiometer, Copenhagen, Denmark). The P_{CO₂} and P_{O₂} electrodes were calibrated before and after each measurement by humidified and thermostatted gas mixtures delivered by gas mixing pumps (Wösthoff, Bochum, FRG), and the pH electrode was calibrated with precision phosphate buffers (Radiometer, Copenhagen). A fraction of each blood sample was centrifuged and the total CO₂ content of the plasma sample was measured by means of a Capni-Con II Total-CO₂-Analyzer (Cameron Instrument Co., Port Aransas, Texas, USA), which was calibrated with a 20 mmol l⁻¹ standard sodium bicarbonate solution (for details see Nicol, Glass & Heisler, 1983).

Plasma [HCO₃⁻] was calculated as [CO₂]_{total} - α · Pa_{CO₂}, where the solubility of CO₂, α, was derived from the formula reported by Heisler (1984). (Note: the first sign of the last line term of the α-formula is misprinted in Heisler (1984) and should read '+'.)

RESULTS

The arterial P_{CO₂} of lizards exposed to environmental hypercapnia of ≈4% CO₂ increased from about 20 mmHg during normocapnia to about 32 mmHg within 2 h (Fig. 1), causing pH_a to fall from 7.59 to 7.41. Concomitantly, [HCO₃⁻] increased

slightly from 20.8 mmol l^{-1} during normocapnia to 22.7 mmol l^{-1} at 2 h. The apparent plasma buffer value ($-\Delta[\text{HCO}_3^-]/\Delta\text{pH}_a$) calculated from these data is $13 \text{ mequiv l}^{-1} \text{ pH}^{-1}$. During later phases of the experiment, P_{CO_2} remained essentially constant at about 33 mmHg, whereas pH and bicarbonate concentrations rose slowly to the final values of 7.43 and 26.4 mmol l^{-1} , respectively.

Similar changes were observed during exposure to $\approx 7\%$ CO_2 in the environmental gas (Fig. 2). Pa_{CO_2} increased from 20 to 48 mmHg during the first 2 h, which was accompanied by a fall in arterial pH from 7.59 to 7.29. Plasma $[\text{HCO}_3^-]$ rose concomitantly from 21.8 to 27.3 mmol l^{-1} , and the apparent plasma buffer value ($-\Delta[\text{HCO}_3^-]/\Delta\text{pH}_a$), was $17 \text{ mequiv l}^{-1} \text{ pH}^{-1}$. Arterial P_{CO_2} slowly rose to about 53 mmHg. The further increases in pH and bicarbonate concentration to the final values of 7.31 and 30.5 mmol l^{-1} at 72 h of hypercapnia were slower than the time-equivalent rise during 4% CO_2 exposure. The rise in bicarbonate concentration during the time period from 8 to 72 h after onset of 7% CO_2 was insignificant, whereas it was highly significant during 4% CO_2 exposure ($P < 0.01$).

The response of the acid-base status to intra-arterial infusion of bicarbonate was extremely variable among the three individuals. Common features of the response

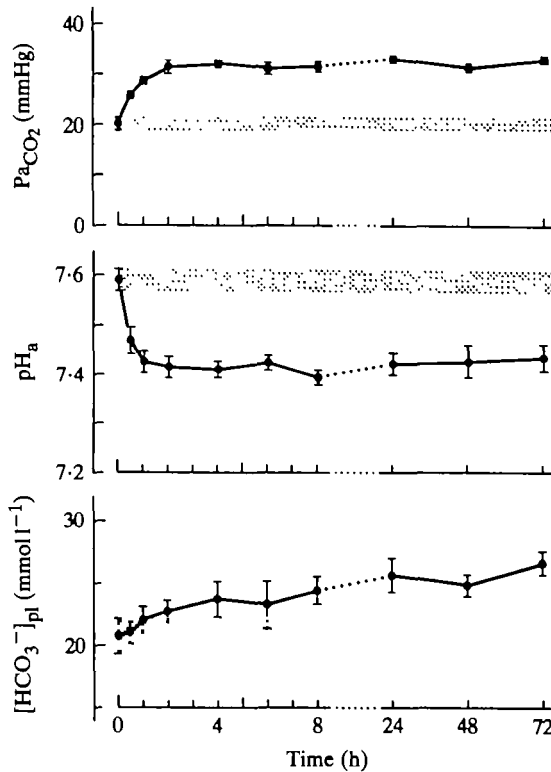


Fig. 1. The effect of hypercapnia on arterial P_{CO_2} , pH and plasma bicarbonate concentration in *Tupinambis* (inspired gas $\approx 4\%$ CO_2 in air). Mean \pm s.e., $N = 7$ (0–8 h), 6 (24 h), 5 (48 and 72 h).

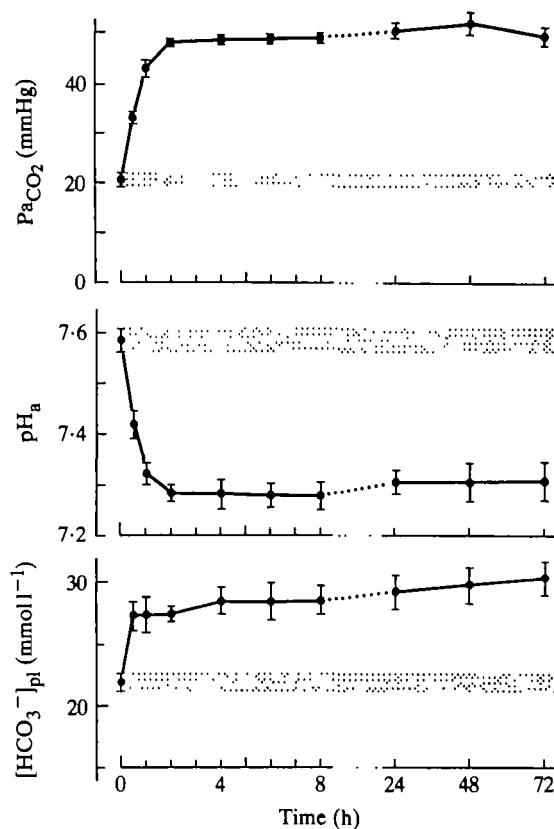


Fig. 2. The effect of hypercapnia on arterial P_{CO_2} , pH and plasma bicarbonate concentration in *Tupinambis* (inspired gas $\approx 7\%$ CO_2 in air). Mean \pm s.e., $N = 8$ (0–8 h), 6 (24 h), 5 (48 and 72 h).

were an essentially unaffected arterial P_{CO_2} , a transient rise in plasma bicarbonate concentration to values higher (by up to 50%) than the pre-infusion levels, and plasma pH restoration close to the prehypercapnia control values. This response, however, was only very transient: plasma pH and $[HCO_3^-]$ gradually decreased again and attained pre-infusion values within the 12 h post-infusion period.

Arterial P_{O_2} was 66 mmHg during normocapnia and was elevated to 79 mmHg after 1 h of $\approx 4\%$ CO_2 . Inhalation of $\approx 7\%$ CO_2 caused a slight fall of P_{aO_2} at 0.5 h, before P_{aO_2} rose to approach 90 mmHg at 2 h. Subsequently, P_{aO_2} gradually decreased in both 4% and 7% CO_2 and finally reattained the range observed during normocapnia (Fig. 3).

DISCUSSION

Exposure of *Tupinambis* to environmental hypercapnia evidently effected a large increase in pulmonary ventilation as indicated by the considerable reduction in the inspired/arterial P_{CO_2} difference. Arterial P_{O_2} , however, rose only transiently and by

a smaller amount. This apparent discrepancy has to be attributed to the different slopes of the oxygen and carbon dioxide dissociation curves, and to the probable occurrence of intracardiac net right to left shunting of partially deoxygenated blood, as observed in the lizard *Varanus exanthematicus* (Heisler *et al.* 1983). Such shunting had only a small effect on arterial P_{CO_2} , but would largely reduce arterial P_{O_2} on the basis of the flat upper part of the oxygen dissociation curve (cf. Glass, Boutilier & Heisler, 1983, 1985).

The effect of hypercapnia on the arterial acid-base status of *Tupinambis* is characterized by rapid increases in plasma P_{CO_2} , which are slowly, but only partially, compensated by elevated bicarbonate concentration and thus result in relatively large changes of plasma pH (Fig. 4):

The initial increases of plasma $[HCO_3^-]$, which occurred within the first 2 h of the hypercapnia experiment, have to be attributed at least partially to action of the extracellular nonbicarbonate buffers. The apparent real plasma buffer values, $\beta = -\Delta[HCO_3^-]/\Delta pH_a$, for the first 2 h were close to the *in vitro* blood buffer value of $18.9 \text{ mequiv l}^{-1} \text{ pH}^{-1}$ reported for the lizard *Varanus niloticus* by Wood & Johansen (1974), although mechanisms other than passive buffering by the blood

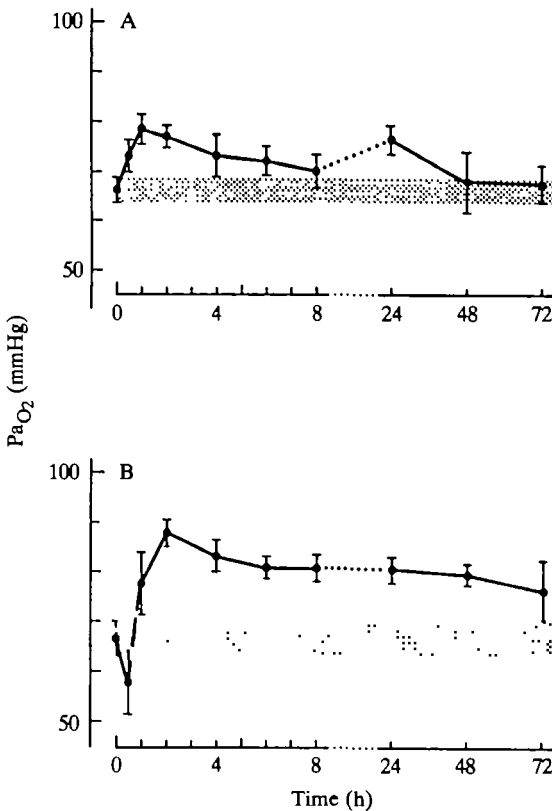


Fig. 3. The effect of different degrees of hypercapnia, (A) $\approx 4\%$ CO_2 ; (B) $\approx 7\%$ CO_2 in the inspired air, on arterial P_{O_2} in *Tupinambis* ($\bar{x} \pm \text{s.e.}$, N as for Fig. 1).

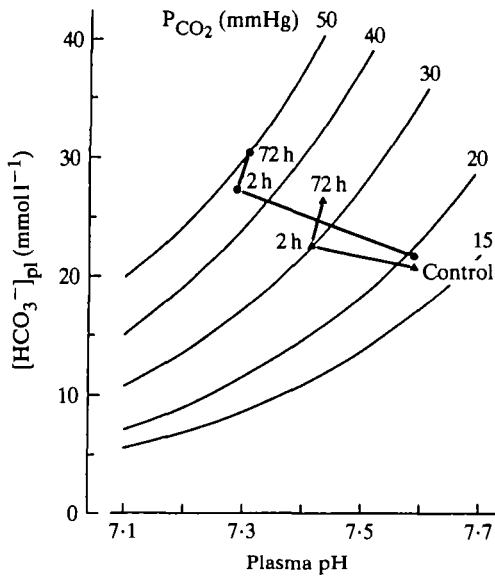


Fig. 4. Presentation of the acid-base status of *Tupinambis* during normocapnia and after 2 and 72 h of hypercapnia displayed in a pH/bicarbonate diagram. pK_1' values determined from measurements of the present study, α_{CO_2} values calculated according to Heisler (1984). (\blacktriangle \approx 4% CO_2 , \bullet \approx 7% CO_2).

(i.e. transfer of bicarbonate-equivalent ions between intracellular and extracellular body compartments) may have influenced the *in vivo* slope (cf. Heisler & Piiper, 1972; Lai, Martin, Attebery & Brown, 1973; Heisler, 1985a). It is, however, impossible to quantify such subcomponents from the present data.

The additional increase of $[HCO_3^-]$ that occurred between 2 and 72 h of hypercapnia has to be attributed to active renal compensation of the rise in P_{CO_2} , since this process took place at essentially constant P_{aCO_2} (Fig. 4). It should be noted that this final change is the same for both 4 and 7% CO_2 . The slightly higher bicarbonate concentration finally attained during 7% CO_2 treatment may be attributed to the slightly higher control values (20.8 vs 21.8 $mmol\ l^{-1}$) and to the initially larger production of bicarbonate by nonbicarbonate buffering in the extracellular compartment.

The extent of the final pH recovery (active and passive components) during both hypercapnic conditions is in the range of 30–40%, i.e. the fall in arterial pH is 30–40% less than that which would have occurred if $[HCO_3^-]$ had remained constant at prehypercapnic control values.

There are few comparable observations from other reptiles, since the effect of hypercapnia has never been studied for longer than a few hours of exposure. Data sets, for normocapnic lizards, however, are consistent with our control measurements during normocapnia (Table 1).

The effects of hypercapnia have been studied in the red-eared turtle *Pseudemys scripta* (Jackson, Palmer & Meadow, 1974). As in other turtle species, the

normocapnic $[\text{HCO}_3^-]$ level is higher than in lizards (cf. Howell & Rahn, 1976), but the increases in $[\text{HCO}_3^-]$ during 3 h of CO_2 inhalation were small, as were those described in *Tupinambis*.

The present data for *Tupinambis* resemble those reported for amphibians, in which the plasma pH compensation during hypercapnia is rather limited (Boutilier, Randall, Shelton & Toews, 1979; Boutilier & Toews, 1981; Toews & Heisler, 1982; Heisler *et al.* 1982; for review see Heisler, 1985c). In particular, the effects of hypercapnia in the toad *Bufo marinus* are strikingly similar to those measured in *Tupinambis*. The total compensation of pH_a after 24 h of 5% CO_2 was 30%, compared to $\approx 33\%$ in *Tupinambis* after 24 h of $\approx 7\%$ CO_2 (Toews & Heisler, 1982). Also, in urodele amphibians the compensation of pH_a during elevated PaCO_2 is small (Boutilier & Toews, 1981) and even absent in *Siren* and *Amphiuma* (Heisler *et al.* 1982).

A small or only limited compensation of plasma pH by accumulation of HCO_3^- in the extracellular compartment is characteristic of air-breathing ectothermic vertebrates. This also applies to air-breathing fishes such as the African lungfish *Protopterus aethiopicus* (DeLaney, Lahiri & Fishman, 1974; DeLaney, Lahiri, Hamilton & Fishman, 1977), which develops a severe respiratory acidosis during aestivation. The recovery of pH_a is slow and only partial. As indicated by the concentration changes of the other plasma electrolytes, the increase in bicarbonate concentration in this species has to be attributed to plasma water loss as a result of desiccation, rather than to bicarbonate gained from active transepithelial ion transfer processes relevant to the acid-base status.

Table 1. Arterial acid-base status of selected reptiles at 25°C

Species	pH	PaCO_2 (mmHg)	$[\text{HCO}_3^-]$ (mmol l ⁻¹)	Reference
Lacertilia				
<i>Tupinambis nigropunctatus</i>	7.59 ± 0.01	20.3 ± 0.7	21.0 ± 0.6	This study
<i>Varanus exanthematicus</i>	7.55	17.1	17.9	Wood, Glass & Johansen (1977)
<i>Sauromalus obesus</i>	7.39	26	14	Crawford & Gatz (1974)
Ophidia				
<i>Coluber constrictor</i>	7.56	18.6	20.1	Nolan & Frankel (1982)
Crocodilia				
<i>Alligator mississippiensis</i>	7.49	17.9	15.2	Davies (1978)
Chelonia				
<i>Chrysemys picta bellii</i>	7.70	29.1	42.7	Nicol, Glass & Heisler (1983)*

For *Tupinambis*: $\bar{x} \pm \text{s.e.}$; $N = 15$.

*The data for *Chrysemys* are interpolated from measurements at 20 and 30°C. The plasma bicarbonate concentrations were directly measured as in the present study, whereas the values from the other studies are indirectly determined from pH_a and PaCO_2 .

The tropical freshwater teleost *Synbranchus marmoratus* lacks any compensation of the rise in P_{CO_2} from about 6 mmHg during water-breathing to 26 mmHg during air-breathing. Plasma pH is reduced accordingly without any persistent elevation of plasma $[\text{HCO}_3^-]$ (Heisler, 1982).

In contrast to air-breathing ectotherms, exclusively water-breathing fish compensate hypercapnic disturbances of the acid–base status almost completely by large increases of plasma $[\text{HCO}_3^-]$ (for reviews see Heisler, 1984, 1985*b*). This compensation of plasma pH during hypercapnia is particularly fast and complete in marine fish including both elasmobranchs (e.g. Heisler *et al.* 1976) and teleosts (e.g. Toews *et al.* 1983).

The striking differences between air- and water-breathing ectothermic vertebrates in their response to hypercapnic acid–base disturbances are correlated with differences in the pattern of the normocapnic acid–base status. Aerial respiration is usually coupled with considerably higher values for P_{aCO_2} and plasma $[\text{HCO}_3^-]$ than are found in water-breathing animals. In contrast, plasma pH is very similar in these two groups of animals (Robin, Bromberg & Cross, 1969; Rahn, Wangenstein & Farhi, 1971; Dejours, 1975; Heisler, 1982).

Limited compensation of hypercapnia in air-breathing lower vertebrates may be attributed to a number of factors. As can easily be derived from the Henderson–Hasselbalch equation, if the plasma bicarbonate concentration is at a higher level during normocapnia, then much larger quantities of bicarbonate must be accumulated in order to compensate a given rise in P_{CO_2} . These larger quantities, however, may not be available to the animals, or the accumulation of these amounts may cause disturbances in the electrolyte balance which are too large to be tolerated (cf. Heisler, 1982). The almost fivefold rise of P_{CO_2} in *Synbranchus* during transition from water- to air-breathing, for example, would require elevation of the bicarbonate concentration from 24 to 111 mmol l^{-1} for complete restoration of plasma pH (cf. Heisler, 1982). Such elevation would imply reduction of the $[\text{Cl}^-]$ to values close to zero, which certainly interferes with electrophysiological mechanisms (e.g. Tauc & Gerschenfeld, 1961; Strumwasser, 1962; see also Eccles, 1964).

The availability of bicarbonate, and the electrolyte disturbances induced by bicarbonate accumulation, however, are apparently not the ultimate factors to limit extracellular pH compensation in air breathers. When all available data on the acid–base regulation during hypercapnia are critically reviewed, it becomes evident that certain plasma bicarbonate concentrations are never surpassed, irrespective of the extent of hypercapnia (Heisler, 1985*c*). These thresholds are species-specific, but seem to vary to only a limited extent within animal classes. For fish the threshold is in the range of 22–27 mmol l^{-1} , for amphibians 22–33 mmol l^{-1} , and for higher vertebrates 40–50 mmol l^{-1} (Heisler, 1985*c*).

The data obtained for *Tupinambis* in the present study are in accordance with these threshold values of other animal classes. *Tupinambis* does not increase the plasma bicarbonate concentration, even after 72 h of hypercapnia, beyond about 30 mmol l^{-1} , and further demands on the acid–base regulatory system by exposure to higher concentrations of CO_2 do not significantly elevate plasma $[\text{HCO}_3^-]$.

The net gain of bicarbonate by excretion of H^+ ions to, or direct uptake of HCO_3^- from, the environment is limited by various factors in fishes and amphibians (Heisler, 1985*b,c*). It could be argued that factors such as insufficient environmental ion concentrations would be responsible for the observed limitation in the elevation of plasma bicarbonate concentration. It was demonstrated, however, that gradual increases in sodium and bicarbonate concentration in the environmental water of the anuran amphibian *Siren* (Heisler *et al.* 1982) and of the freshwater teleost fish *Cyprinus* (Claiborne & Heisler, 1984) did not enhance the compensation of hypercapnia. Also, direct infusion into these animals raised plasma bicarbonate concentration only transiently, before the bicarbonate was quantitatively excreted to the environmental water.

Although terrestrial species such as *Tupinambis* gain bicarbonate by renal mechanisms, which are independent of environmental factors, the rate of bicarbonate gain could be too small to result in higher bicarbonate concentration values than observed during the experimental time period of the present study. In *Tupinambis* intra-arterial infusion of bicarbonate, however, resulted in an only transient increase of plasma bicarbonate concentration, before the original values were restored.

We conclude that the threshold for the mechanisms for retaining and resorbing bicarbonate in the reptile *Tupinambis* is similar to that described for other classes of animals. This threshold is responsible for the loss of infused bicarbonate when the maximal level has been attained, and the observed rather poor compensation of hypercapnic acid-base disturbances in this species.

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