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α-Adrenergic regulation of systemic peripheral resistance and blood flow distribution in the turtle *Trachemys scripta* during anoxic submergence at 5°C and 21°C

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Summary

Anoxic exposure in the anoxia-tolerant freshwater turtle is attended by substantial decreases in heart rate and blood flows, but systemic blood pressure (P_{sys}) only decreases marginally due to an increase in systemic peripheral resistance (R_{sys}). Here, we investigate the role of the α-adrenergic system in modulating R_{sys} during anoxia at 5°C and 21°C in the turtle *Trachemys scripta*, and also describe how anoxia affects relative systemic blood flow distribution (%$\dot{Q}_{sys}$) and absolute tissue blood flows. Turtles were instrumented with an arterial cannula for measurement of P_{sys} and ultrasonic flow probes on major systemic blood vessels for determination of systemic cardiac output ($\dot{Q}_{sys}$). α-Adrenergic tone was assessed from vascular injections of α-adrenergic agonists and antagonists (phenylephrine and phentolamine, respectively) during normoxia and following either 6 h (21°C) or 12 days (5°C) of anoxic submergence. Coloured microspheres, injected through a left atrial cannula during normoxia and anoxia, as well as after α-adrenergic stimulation and blockade during anoxia at both temperatures, were used to determine relative and absolute tissue blood flows.

Anoxia was associated with an increased R_{sys} and functional α-adrenergic vasoactivity at both acclimation temperatures. However, while anoxia at 21°C was associated with a high systemic α-adrenergic tone, the progressive increase of R_{sys} at 5°C was not mediated by α-adrenergic control. A redistribution of blood flow away from ancillary vascular beds towards more vital circulations occurred with anoxia at both acclimation temperatures. %$\dot{Q}_{sys}$ and absolute blood flow were reduced to the digestive and urogenital tissues (approximately 2- to 15-fold), while %$\dot{Q}_{sys}$ and absolute blood flows to the heart and brain were maintained at normoxic levels. The importance of liver and muscle glycogen stores in fueling anaerobic metabolism were indicated by increases in %$\dot{Q}_{sys}$ to the muscle at 21°C (1.3-fold) and liver at 5°C (1.7-fold). As well, the crucial importance of the turtle shell as a buffer reserve during anoxic submergence was indicated by 40–50% of $\dot{Q}_{sys}$ being directed towards the shell during anoxia at both 5°C and 21°C. α-Adrenergic stimulation and blockade during anoxia caused few changes in %$\dot{Q}_{sys}$ and absolute tissue blood flow. However, there was evidence of α-adrenergic vasoactivity contributing to blood flow regulation to the liver and shell during anoxic submergence at 5°C.

Key words: red-eared slider, *Trachemys scripta*, anoxia, temperature, cardiovascular, systemic resistance, cardiac output, blood pressure, α-adrenergic control, microsphere, blood flow distribution.

Introduction

Freshwater turtles, particularly those of the genera *Chrysemys* and *Trachemys*, exhibit a remarkable ability to survive prolonged periods of anoxia (Johlin and Moreland, 1933; Jackson and Ultsch, 1982; Ultsch and Jackson, 1982; Herbert and Jackson, 1985a,b; Hicks and Farrell, 2000a). At 3°C, these turtles recover physiological functions after 12 weeks of anoxia and survive for up to 5 months of anoxia (Ultsch and Jackson, 1982; Herbert and Jackson, 1985b). This is achieved by metabolic depression, where biochemical and metabolic adaptations balance anaerobic energy production to the reduced demand, and a large capacity of the shell to buffer the ensuing metabolic acidosis and elevated lactate levels (Jackson and Schmidt-Nielsen, 1966; Jackson, 1968, 2000, 2002; Herbert and Jackson, 1985b; Storey, 1996; Lutz and Storey, 1997).

The cardiovascular system continues operating during anoxia to transport metabolites between tissues, but because of the body's low metabolic demand and direct effects of reduced oxygen on the myocardium, both heart rate (f_H) and systemic blood flow ($\dot{Q}_{sys}$) are greatly depressed (Herbert and Jackson, 1985b; Hicks and Wang, 1998; Hicks and Farrell, 2000a). While systemic blood pressure (P_{sys}) also decreases during

anoxia, the reduction in $\dot{Q}_{sys}$ is considerably larger, indicating a substantial (three- to fivefold) increase in systemic peripheral resistance (R_{sys}) (Hicks and Farrell, 2000a). The basis of this augmented R_{sys}, which maintains the systemic circulation in a state of hypotension during anoxia, remains unidentified (Hicks and Farrell, 2000b).

In most vertebrates, R_{sys} is predominantly controlled by α-adrenergic innervation of the resistance vessels, and α-adrenergic mediated peripheral vasoconstriction occurs during hypoxia in many species (Lillo, 1979; Fritsche and Nilsson, 1989; Axelsson and Fritsche, 1991; J. A. W. Stecyk and A. P. Farrell, manuscript submitted for publication). Likewise, systemic α-adrenergic tone mediates the increased peripheral vasoconstriction that occurs during diving in mammals and birds (Butler and Jones, 1971; Butler, 1982; Lacombe and Jones, 1991; Signore and Jones, 1995). In freshwater turtles, adrenergic fibres innervate the systemic circulation (Berger and Burnstock, 1979), and α-adrenergic stimulation increases R_{sys} in normoxic animals (Comeau and Hicks, 1994; Hicks and Farrell, 2000b; Overgaard et al., 2002). Anoxic exposure of turtles is associated with high concentrations of circulating catecholamines and it is possible that they increase R_{sys} through arteriolar α-adrenergic stimulation (Wasser and Jackson, 1991; Keiver and Hochachka, 1991; Keiver et al., 1992). However, α-adrenergic regulation of the cardiovascular system seems depressed during anoxia, as the stimulatory effects of adrenaline on f_H and P_{sys} are blunted during anoxia at both cold and warm temperatures, and this is correlated with a reduced density of β-adrenergic receptors on the heart (Hicks and Wang, 1998; Hicks and Farrell, 2000b). The α-adrenergic control of vasomotor tone during anoxia has not been directly investigated in turtles. Consequently, it remains unknown whether the high levels of circulating catecholamines saturate the α-adrenergic receptors, so that exogenous application does not affect R_{sys}, or whether α-adrenergic control of vasomotor tone is suppressed during anoxia. Thus, our first objective was to test the hypothesis that increased α-adrenergic tone accounts for the augmented R_{sys} during anoxia in the turtle *Trachemys scripta*. We examined the α-adrenergic regulation of $\dot{Q}_{sys}$, P_{sys} and R_{sys} with injections of α-adrenergic agonists and antagonists in normoxic and anoxic turtles acclimated to 5°C or 21°C.

The increase in R_{sys} with hypoxia in vertebrate species is usually accompanied by a redistribution of systemic blood flow that reflects differences in metabolic needs among tissues, with critical systems, such as the brain and the heart, receiving a high priority to prevent damage from anoxia. For example, a high priority to cerebral blood flow has been observed in fish that are tolerant to oxygen shortage during both anoxia and severe hypoxia (Nilsson et al., 1994; Yoshikawa et al., 1995; Söderström et al., 1999). Similarly, during hypoxia and underwater diving, endotherms redistribute blood flow towards cerebral, myocardial and adrenal vascular beds, while blood flow to visceral organs is reduced by a selective vasoconstriction which, in many cases, is mediated by α-adrenergic control (Johansen, 1964; Elsner et al., 1966; Chalmers et al., 1967; Krasney, 1971; Butler and Jones, 1971; Jones et al., 1979; Zapol et al., 1979). Consistent with these blood flow patterns, blood flow to various visceral organs is reduced during short-term anoxia in anaesthetized turtles, while brain blood flow is largely maintained (Davies, 1989, 1991; Bickler, 1992; Hylland et al., 1994, 1996).

The redistribution of blood flow during short-term anoxia in anaesthetized turtles (Davies, 1989) indicates that the different vascular beds respond differently to hypoxia. However, the anaesthetized turtle does not exhibit the otherwise well-documented depression of cardiac activity during anoxia, which may reflect the complex effects of anaesthetics on the heart and local blood flow regulation (e.g. Smith and Wollman, 1972; Marcus et al., 1976). Therefore, it is uncertain whether these findings can be applied to unanaesthetized animals and to a prolonged period of anoxia that can occur naturally. Thus, our second objective was to identify which critical tissues receive a high priority of blood flow during prolonged anoxia when cardiovascular function is depressed. Relative systemic blood flow distribution and absolute tissue blood flows were determined during normoxia and anoxia by the injection of coloured microspheres, while simultaneously measuring absolute blood flows in the major arteries. Microspheres were also injected following α-adrenergic stimulation and blockade during anoxia to determine α-adrenergic control of blood flow redistribution.

Materials and methods

Experimental animals

Twenty-six red-eared sliders *Trachemys scripta* Gray, body mass 0.45–1.9 kg (1.20±0.07 kg, mean ± S.E.M.) were used in this study. Turtles were obtained from Lemberger Inc. (Oshkosh, WI, USA) and airfreighted to Aarhus University (Denmark), where they were maintained for several months before experimentation. The turtles studied at 21°C were held in large fibreglass aquaria under a 14 h:10 h L:D photoperiod, had free access to dry basking platforms under infrared lamps to allow behavioural thermoregulation and were fed dead fish several times a week. Food was withheld during the experimentation period. The turtles studied at 5°C were kept in a large flow-through polypropylene tank at 5°C with access to air for 6 weeks prior to instrumentation. All 5°C turtles were fasted during this period. Experiments were performed between February and April 2001 and all procedures were in accordance with the laws of animal care and experimentation in Denmark.

Surgical procedures

Turtles were intubated with soft rubber tubing for artificial ventilation with isoflurane (4% in room air prepared by a Halothane vaporizer; Dräger, Lubeck, Germany) at a rate of 8–15 breaths min^{-1} and a tidal volume of 10–20 ml kg^{-1} using a Harvard Apparatus Ventilator (HI 665, Harvard Apparatus Inc., Holliston, MA, USA). Once a surgical plain of anaesthesia was achieved, as determined by the lack of a pedal

withdrawal reflex, the isoflurane level was reduced to either 0.5% or 1% and maintained at this level throughout the operation, which lasted approximately 40 min.

For placement of catheters and flow probes, the heart and central vascular blood vessels were accessed by excision of a 3 cm×4 cm piece of the plastron using a bone saw. An occlusive catheter (PE-50 containing saline with 100 i.u. ml^{-1} heparin) was advanced from the left thyroid artery into the right subclavian artery originating from the right aortic arch. For blood flow measurements, 1.0–1.5 cm sections of major systemic blood vessels were freed from the surrounding connective tissue for placement of ultrasonic blood flow probes (sizes 2–3 mm; Transonic Systems Inc., Ithaca, NY, USA). Turtles exposed to anoxia were instrumented with flow probes around the left aortic arch (LAo), the right aortic arch (RAo), and a single probe around both the left subclavian and left carotid arteries (Lsubcar). The use of one flow probe for monitoring blood flow through two vessels has previously been validated in turtles (Wang and Hicks, 1996; see also Akagi et al., 1987). In addition, the left atrium of these animals was cannulated for the injection of coloured microspheres into the systemic circulation. A PE-90 catheter, flared at the end to prevent withdrawal, was inserted through a 0.3 cm incision of the atrial wall into the lumen and fastened to the atrial wall by surgical silk (4-0). The pericardium was subsequently closed with two or three sutures (4-0 surgical silk). Control normoxic turtles were instrumented with a single flow probe around the LAo, and $\dot{Q}_{sys}$ was determined from the equation $\dot{Q}_{sys}=2.8\times\dot{Q}_{LAo}$, as previously verified (Comeau and Hicks, 1994; Wang and Hicks, 1996). Acoustic gel was infused between the blood vessels and flow probes to enhance the signal and the excised piece of the plastron was resealed in its original position using surgical tape and fast-drying epoxy resin.

After completion of the operation, turtles were ventilated with room air until they resumed spontaneous ventilation. Turtles were then allowed to recover in individual water-filled aquaria (40 cm×30 cm×30 cm), covered with black plastic to minimize visual disturbance, for either 48 h at 21°C or 72 h at 5°C.

Experimental protocol

All experiments were performed on unrestrained turtles that were free to move within the aquaria. Prior to any experimental manipulation, arterial blood samples were obtained through the arterial cannula for the measurement of hematocrit and arterial pH. Turtles studied during anoxia were denied air access while the aquarium water was continuously bubbled with N_2 (water P_{O_2} <0.3 kPa) and were exposed to anoxia for either 6 h at 21°C or 12 days at 5°C. Normoxic turtles had free access to room air throughout the experimentation period.

Injections of α-adrenergic agonists and antagonists were used to examine the α-adrenergic regulation of R_{sys}. Anoxic turtles were treated sequentially with the α-adrenergic agonist phenylephrine (5 μg kg^{-1} and subsequently 50 μg kg^{-1}), the α-adrenergic antagonist phentolamine (3 mg kg^{-1}) and, finally, phenylephrine (50 μg kg^{-1}). After injections of phenylephrine, cardiovascular variables were allowed to return to baseline values before continuing the protocol. Following phentolamine injection, cardiovascular function was allowed to stabilize before subsequent drug injections. Control normoxic turtles were only treated with phentolamine (3 mg kg^{-1}). All chemicals, purchased from Sigma-Aldrich, Denmark, were dissolved in physiological turtle saline (in mmol l^{-1}: NaCl, 105; KCl, 2.5; $CaCl_2$, 1.3; $MgSO_4$, 1; $NaHCO_3$, 15; NaH_2PO_4, 1; pH 7.8) and injected as a single 0.5–1.0 ml bolus through the arterial cannula, which was subsequently flushed with saline. The total volume injected never exceeded 2 ml and control saline injections of 2 ml did not cause haemodynamic changes.

Coloured polystyrene microspheres (25 μm in diameter) (Dye Track, Triton Technologies, San Diego, CA, USA) were used to measure regional blood flow distribution. Microspheres, suspended in the manufacturer supplied saline, which contained 0.05% Tween 80 to prevent agglomeration and 0.01% Thimerosal to act as a bacteriostat, to a final concentration of 4.0×10^5 spheres ml^{-1} were injected into the anoxic exposed group of turtles in 1 ml portions through the left atrial cannula. This procedure was conducted during normoxia while the animals were not ventilating their lungs (tangerine microspheres), during anoxia (orange microspheres), during the maximal haemodynamic response to the first 50 μg kg^{-1} phenylephrine injection (lemon microspheres) and, finally, after subsequent injection of phentolamine (canary microspheres), when cardiovascular function stabilized. A minimum of 30 min was allowed between microsphere injection and any subsequent experimental manipulation. To minimize conglomeration of the microspheres in the heart, the microsphere solution was sonicated for 1 min and vortexed for 30 s immediately before injection. After microsphere delivery, which lasted approximately 1 min, the syringe and cannula were flushed twice (0.75 ml each time) with physiological turtle saline and rinsed four times with acidified ethanol (0.2% v:v HCl, 37% ethanol) and the liquid retained such that the number of spheres remaining in the syringe could be determined. A reference blood sample (0.3–0.5 ml) was taken from the arterial cannula 20 min after the normoxic and anoxic microsphere injections to assess whether the microspheres had indeed been trapped in the tissues.

At the completion of the protocol, turtles were euthanized with a vascular injection of pentobarbital (100 μg kg^{-1}). All animals were then dissected to separate organs and tissues, including the integument, red and white muscle, bones, esophagus, stomach, intestines (including pancreas), spleen, ventricle, atria, brain, liver (including gallbladder), kidneys, gonads, fat, connective tissue (included major blood vessels, bladder and thyroid gland) and eyes, which were cut into 7–15 g pieces and placed in individual polypropylene conical centrifuge tubes. The shell was subsampled, using seven representative samples of 1.0–8.9 g (mean 3.8±0.2 g) and representing 6.9±0.4% of total shell mass. Three samples were

taken from the plastron, one from each of the anterior, medial and posterior sections, and four from the carapace, one from each side of the shell (costal scutes) and two from the vertebral scutes, one anterior and one posterior. Tissue samples were stored at room temperature for up to 8 weeks, allowing for unaided tissue degradation to occur, before chemical digestion and microsphere recovery from the tissues.

Tissue digestion and microsphere recovery

Immediately prior to tissue digestion, 3000 control spheres (blue, 25 μm diameter), suspended in saline containing 0.05% Tween 80 and 0.01% Thimerosal, were added to each sample to evaluate the efficiency and quality of the recovery process. Extraction of microspheres from reference blood samples and soft tissues was as follows:

(1) 'Alkaline digesting reagent' (2 mol l^{-1} KOH; 3–5× tissue volume) was added to the polypropylene tubes and the tissue left to digest for 24 h. During digestion, the tubes were maintained at 60°C and intermittently vortexed and sonicated for 45 s. After sonication, the probe-tip was rinsed into the sample tube with acidified ethanol.

(2) After digestion, tubes were filled to capacity with 60°C distilled water and the contents mixed by repeated inversion. Samples were then centrifuged at 1500 ***g*** for 15 min and the supernatant aspirated to a level safely above the visible pellet.

(3) The pellet was resuspended by sonication in 15% Triton-X solution (v:v Triton-X:distilled water, 0.1 g l^{-1} sodium azide; 3–5× original tissue volume) heated to 60°C. The tubes were then centrifuged for 5 min at 1500 ***g*** and the supernatant aspirated without disturbing the pellet. If the pellet was large after the Triton-X wash, the protocol was repeated from step 1 until the pellet was no longer visible.

(4) The non-visible pellet was resuspended by sonication in 2–4× the original tissue volume of acidified ethanol, and again centrifuged for 5 min at 1500 ***g*** and the supernatant safely aspirated. Finally, the acidified ethanol wash was repeated, and after decanting, the remaining supernatant was left to evaporate at room temperature.

Microsphere recovery from bone and shell samples followed the same protocol as described above, but included two additional steps. After step 3, the pellet was resuspended by sonication in approximately 5× the original tissue volume of 'bone digesting reagent' (0.12% EDTA, 5.38% HCl, 94% H_2O). Tubes were then maintained at 60°C for at least 12 h with intermittent sonication, centrifuged for 5 min at 1500 ***g*** and aspirated to a safe level. The procedure was repeated if bone fragments remained following centrifugation. Once the bone or shell was completely dissolved, the remaining pellet was resuspended by sonication in 15% Triton-X solution and the protocol described above was followed (i.e. alkaline digestion if a large pellet remained, or two acidified ethanol washes if the pellet was not visible).

Measurement of microsphere distribution and terminology

Following tissue digestion, 250 μl of 2-(2-ethoxyethoxy)ethylacetate was added to each tube and vortexed to extract the dye from the microspheres. The dye was allowed to extract for 20 min and then centrifuged at 1500 g for 5 min. 200 μl of the supernatant was then transferred to a microplate and the absorption of each sample was measured at five wavelengths with a microplate spectrophotometer (Molecular Devices Corporation, Sunnyvale, California, USA) referenced to 200 μl of 2-(2-ethoxyethoxy)ethylacetate. The five wavelengths used were those of maximal absorbance for each microsphere colour label, namely 390 nm (lemon), 440 nm (canary), 495 nm (orange), 525 nm (tangerine) and 672 nm (blue). Samples with absorbance readings greater than 1.3 units were diluted and reanalyzed to ensure linearity between absorbance and dye concentration. Correction for spectral overlap among the five colour labels and subsequent determination of the number of each colour of microsphere per sample was resolved through a matrix conversion computer program (Triton Technologies Inc., San Diego, CA, USA).

The total number of each colour of microsphere recovered per animal was determined as the sum of all microspheres recovered and those estimated to be trapped in the non-digested portion of the shell (the number of microspheres recovered from each of the representative shell samples did not differ statistically, so the amount of microspheres in the non-digested portion of the shell was assumed to be identical). Recovery of injected microspheres was expressed relative to the number of microspheres injected (i.e. 4.0×10^5 minus the number of spheres remaining in the syringe after injection). The fraction of systemic cardiac output (%$\dot{Q}_{sys}$) directed to each tissue was calculated as the quotient of the number of microspheres recovered per tissue and total microspheres recovered from systemic tissues. Absolute blood flows (μl min^{-1} g^{-1}) to systemic tissues were calculated by multiplying %$\dot{Q}_{sys}$ for each tissue by systemic cardiac output ($\dot{Q}_{sys}$), as measured by the ultrasonic flow probes (see below).

Calculation of haematological and cardiovascular variables

Hematocrit was determined as the fractional erythrocyte volume in a capillary tube following 3 min of centrifugation at 10 000 ***g***. Arterial pH was measured using a Radiometer pH electrode (PS-1 204, Copenhagen, Denmark) maintained and calibrated at the acclimation temperature of the experimental animal in a BMS Mk3 electrode set-up. Electrode output was displayed on a Radiometer PHM 73 pH monitor. Systemic blood pressure (P_{sys}) was measured by attaching the arterial cannula to a disposable pressure transducer (Baxter Edward, model PX600, Irvine, CA, USA) calibrated daily against a static water column. The signal from the pressure transducer was amplified using an in-house built preamplifier. Flow probes were connected to two, dual-channel blood flow meters (T201, Transonic Systems Inc., Ithaca, NY, USA). All signals were continuously recorded with a Biopac MP100 computer assisted data acquisition system (Biopac Systems Inc., Goleta, CA, USA) at 50 Hz and data recordings were analysed offline using AcqKnowledge data analysis software (version 3.5.7; Biopac Systems Inc., Goleta, CA, USA).

Systemic cardiac output ($\dot{Q}_{sys}$) was calculated as

$\dot{Q}_{LAo}+\dot{Q}_{RAo}+2\dot{Q}_{Lsubcar}$ for the anoxic exposed turtles, whereas $\dot{Q}_{sys}$ was estimated as $2.8\times\dot{Q}_{LAo}$ for the control normoxic turtles. Heart rate (f_H) was derived from the beat-to-beat interval of the P_{sys} trace. Systemic stroke volume (V_{Ssys}) was calculated as $\dot{Q}_{sys}/f_H$ and systemic resistance (R_{sys}) as $P_{sys}/\dot{Q}_{sys}$ with the assumption that right atrial pressure is negligible. Systemic power output (PO_{sys}) was calculated as $\dot{Q}_{sys}\times P_{sys}/M_v$, where P_{sys} is measured in kPa and M_v is ventricular mass (g).

Data analysis and statistics

Normoxic control values for all experimental groups were recorded for a 30–60 min period immediately before drug injections or anoxic exposure. At 21°C, haemodynamic variables were recorded continuously throughout the 6 h anoxic period; reported haemodynamic values from this time period were averaged from 5 min periods at each hour of anoxic exposure. At 5°C, haemodynamic variables were recorded for 30 min on days 3, 8 and 12 of the anoxic exposure and continuously throughout the period of drug and microsphere injections on day 12. At both temperatures, we report the maximal response following injections of the α-adrenergic agonist phenylephrine, which normally occurred within 5 min and 15 min after injection at 21°C and 5°C, respectively, while new steady state values were attained 0.5 h and 1 h after injection of the α-adrenergic antagonist phentolamine at 21°C and 5°C, respectively.

Values presented for all haematological and cardiovascular variables, $\%\dot{Q}_{sys}$ and absolute tissue blood flows at each sample time are means ± S.E.M. Within-group comparisons of cardiovascular variables were determined using a one-way repeated measures (RM) analysis of variance (ANOVA), and comparisons of cardiovascular variables between acclimation temperatures were performed using a *t*-test. Similarly, comparisons of $\%\dot{Q}_{sys}$ and absolute tissue blood flow between acclimation temperatures were performed using *t*-tests. Changes in $\%\dot{Q}_{sys}$ between routine normoxic and routine anoxic conditions, as well as following α-adrenergic stimulation and blockade, were determined using a two-way RM-ANOVA on arcsine-transformed $\%\dot{Q}_{sys}$ data, with tissue $\%\dot{Q}_{sys}$ and condition as the two factors. Changes in absolute blood flow to different tissues during normoxic control, anoxic control, and following the α-adrenergic agonist and antagonist drug injections, were assessed with a one-way RM-ANOVA unless the data were not normally distributed, in which case a Friedman RM-ANOVA on ranks was used. Where appropriate, multiple comparisons were performed using Student–Newman–Keuls tests and in all instances significance was accepted when $P<0.05$.

Results

Accuracy of microsphere technique

Accurate use of microspheres requires that several criteria be satisfied. Foremost, it must be assumed that all the microspheres are trapped in the capillary beds during their first passage through the circulation. Complete trapping of microspheres during normoxia and anoxia was confirmed by the lack of microspheres in arterial blood samples withdrawn 20 min after injections.

Secondly, injected microspheres must be adequately mixed with blood at the injection site to provide a homogenous solution such that the same concentration of microspheres prevails at all arterial sites. We injected the microspheres into the left atrium, and while atrial and ventricular contraction are likely to have ensured good mixing, the undivided ventricle of turtles allows for some of the injected microspheres to be shunted into the pulmonary circulations (White et al., 1989). During normoxia, we therefore injected the microspheres during breath-hold, when pulmonary blood flow and Left-to-Right shunting is low (e.g. White et al., 1989; Hicks, 1994; Wang and Hicks, 1996). During anoxia, at least at warm temperatures, pulmonary blood flow is greatly reduced due to hypoxic pulmonary vasoconstriction (Hicks and Wang, 1998; Crossley et al., 1998). These precautions seemed effective as the relative number of spheres recovered from the lungs was low (5.2±1.1%; $N=44$) and did not change with either injection time or acclimation temperature.

The coloured microsphere technique also relies on an efficient microsphere isolation and purification protocol such that measured absorbance intensities correlate directly with the number of microspheres. Indeed, analysis of reference microsphere samples in the present study revealed linear relationships ($P<0.001$ in all cases) between microsphere number and measured absorbance for each of the five colour labels (blue, $y=0.0002x$, $r^2=0.93$; tangerine, $y=0.0022x$, $r^2=0.98$; orange, $y=0.0004x$, $r^2=0.96$; canary, $y=0.0006x$, $r^2=0.98$; lemon, $y=0.0003x$, $r^2=0.87$). Furthermore, there was a linear relationship ($P<0.001$ in all cases) between the number of microspheres determined through matrix conversion and the number of microspheres in each experimental sample for all five colour labels (blue, $y=0.9712x$, $r^2=0.93$; tangerine, $y=0.9873x$, $r^2=0.98$; orange, $y=1.6673x$, $r^2=0.96$; canary, $y=1.2674x$, $r^2=0.98$; lemon, $y=1.3199x$, $r^2=0.93$). Further, tissue type did not influence the efficiency of microsphere recovery. The relative recovery of blue control spheres (73±2%; $N=1215$), used to assess microsphere loss during the extraction procedure, did not differ between tissue types. Relative recovery of injected microspheres differed among the different microsphere colour labels. Tangerine coloured spheres (injected at normoxic control) had a recovery of 29±5%, whereas the lemon coloured spheres (injected after phenylephrine during anoxia) had a recovery of 140±2%. 74±13% of the canary (injected after phentolamine during anoxia) and 103±10% of the orange (routine anoxic injection) microspheres were recovered.

The accuracy of the microsphere technique for determination of $\%\dot{Q}_{sys}$ can be quantified in the present study because $\dot{Q}_{sys}$ was measured simultaneously with blood flow probes (Fig. 1). $\%\dot{Q}_{sys}$ to tissues perfused by the left subclavian (left foreleg integument, bone and muscle) and carotid arteries (head and neck bones, integument, muscle, esophagus, trachea, thyroid, brain and eyes) compared to the relative blood flow in

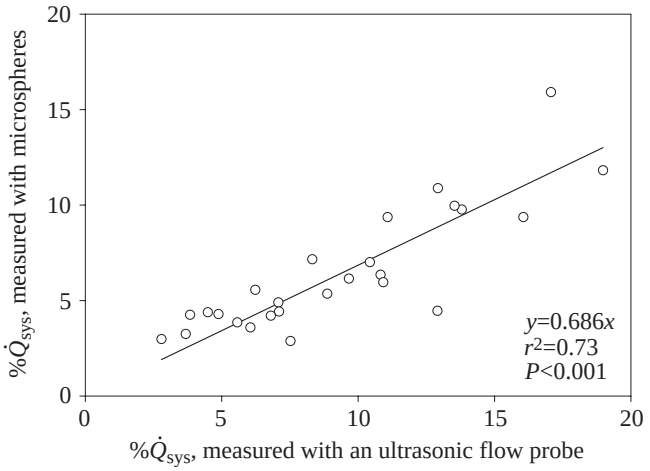

Fig. 1. Regression plot of percent of total systemic cardiac output (%$\dot{Q}_{sys}$) directed to tissues perfused by the left subclavian and carotid arteries, as measured with ultrasonic flow probes (x axis) and the microsphere recovery technique (y axis). Values presented are from 5°C and 21°C acclimated turtles (N=7) during normoxic control, routine anoxia and following α-adrenergic stimulation and blockade during anoxia.

these vessels were linearly related, but the microsphere technique underestimated %$\dot{Q}_{sys}$ by approximately 30%.

Blood flow ratios among the major systemic blood vessels

Blood flows in all major systemic arteries, with the exception of the right carotid and right subclavian arteries, were successfully measured in 9 of the 14 turtles exposed to anoxia and instrumented with three ultrasonic flow probes. Assuming that these vessels receive the same flows as the left carotid and left subclavian arteries, we estimate that $\dot{Q}_{LAo}$, $\dot{Q}_{RAo}$ and $\dot{Q}_{Lsubcar}$ account for 36.3±0.01%, 35.9±0.01% and 13.9±0.01%, respectively (N=9 in all cases), of $\dot{Q}_{sys}$ in turtles. This proportional distribution was not affected by acclimation temperature, oxygen availability (Table 1) or injections of phenylephrine and phentolamine during anoxia (data not shown).

Effect of acclimation temperature on normoxic haematological variables and α-adrenergic control of cardiovascular function

Normoxic hematocrit did not vary significantly between acclimation temperatures, but arterial pH was significantly greater in 5°C normoxic turtles than 21°C normoxic turtles (Table 2). Cold-acclimation resulted in large reductions in f_H and $\dot{Q}_{sys}$, while R_{sys} increased significantly (Table 3). $\dot{Q}_{sys}$, f_H and PO_{sys} were 5–11× lower at 5°C than at 21°C, with respective Q_{10} values of 2.6, 2.8 and 4.7. In contrast, R_{sys} was twofold greater at 5°C than at 21°C. As a result, P_{sys} was reduced by only 46% at 5°C despite a 4.7-fold decrease in $\dot{Q}_{sys}$.

α-Adrenergic regulation of cardiovascular function differed between acclimation temperatures in normoxic turtles (Table 3). At 21°C, injection of the α-adrenergic antagonist phentolamine did not affect any of the measured haemodynamic variables, whereas injection at 5°C

Table 1. *Blood flows through select major systemic arches of 5°C- and 21°C-acclimated turtles during normoxic and anoxic exposure*

Acclimation temperature	Condition	Variable	Blood flow (ml min^{-1} kg^{-1})	%$\dot{Q}_{sys}$	Factor*
5°C	Normoxia	$\dot{Q}_{sys}$	11.3±1.4		
		$\dot{Q}_{LAo}$	3.9±0.4	34.8±0.9	2.88±0.07
		$\dot{Q}_{RAo}$	4.0±0.7	36.3±4.2	
		$\dot{Q}_{Lsubcar}$	1.7±0.4	14.5±2.2	
	Anoxia	$\dot{Q}_{sys}$	2.68±0.35		
		$\dot{Q}_{LAo}$	0.95±0.06	36.9±3.6	2.79±0.27
		$\dot{Q}_{RAo}$	0.90±0.14	33.3±1.3	
		$\dot{Q}_{Lsubcar}$	0.41±0.09	14.9±1.5	
21°C	Normoxia	$\dot{Q}_{sys}$	46.3±2.8		
		$\dot{Q}_{LAo}$	18.1±2.7	38.6±3.5	2.67±0.16
		$\dot{Q}_{RAo}$	17.2±1.2	37.1±1.5	
		$\dot{Q}_{Lsubcar}$	5.5±0.7	12.1±1.7	
	Anoxia	$\dot{Q}_{sys}$	15.4±2.6		
		$\dot{Q}_{LAo}$	5.5±1.1	34.6±1.9	2.92±0.16
		$\dot{Q}_{RAo}$	5.3±0.5	36.4±3.7	
		$\dot{Q}_{Lsubcar}$	2.3±0.5	14.5±1.1	

*Multiple of $\dot{Q}_{LAo}$ needed to equal $\dot{Q}_{sys}$.

Values are means ± S.E.M. (N=4 at 5°C and 5 at 21°C).

$\dot{Q}_{sys}$, systemic blood flow; $\dot{Q}_{LAo}$, left aortic arch blood flow; $\dot{Q}_{RAo}$, right aortic arch blood flow; $\dot{Q}_{Lsubcar}$, left subcalvian and left common carotid blood flow.

Table 2. *Hematocrit and arterial pH of normoxic turtles*

Acclimation temperature	Hematocrit (%)	Arterial pH
5°C	30±3	7.82±0.05
21°C	21±3	7.53±0.08*

Values are mean ± S.E.M., N=4.

An asterisk indicates significant difference between acclimation temperatures.

significantly reduced R_{sys}, which was manifested as a decrease in P_{sys} and augmented $\dot{Q}_{sys}$ through an increase in V_{Ssys}. Thus, systemic α-adrenergic tone was inversely related with acclimation temperature in normoxic turtles.

Cardiovascular function during anoxia

$\dot{Q}_{sys}$, f_H and P_{sys} were significantly reduced with anoxia at both acclimation temperatures (Figs 2 and 3; Table 4). At 21°C, 2.1-fold and 2.6-fold reductions in f_H and $\dot{Q}_{sys}$, respectively, occurred by 6 h of anoxia and the accompanying fall in P_{sys} led to an almost fourfold reduction in PO_{sys}. Correspondingly, R_{sys} increased 2.3-fold by 6 h of anoxic exposure. At 5°C, the proportional changes in cardiovascular status during anoxia were larger than those observed at 21°C. f_H, $\dot{Q}_{sys}$ and PO_{sys} were reduced by 1.3- to 3.4-fold by day 3 of anoxia, and these initial reductions were then followed by a slower, gradual decline such that by day 12 of anoxia, f_H, $\dot{Q}_{sys}$ and PO_{sys} were maximally reduced by 4.7-fold, 4.3-fold and 6.7-fold, respectively. Similar to the response at 21°C, there was a corresponding increase in R_{sys} (2.9-fold by day 12 of anoxic exposure) at 5°C. However, absolute R_{sys} was 2.3 times greater during 5°C anoxia than 21°C anoxia (Table 4).

Systemic blood flow distribution during normoxia and anoxia

Blood flow distribution differed with acclimation temperature under normoxic conditions (Fig. 4A,C; Table 5), with cold turtles having a significantly higher $\%\dot{Q}_{sys}$ to the integument, but a lower $\%\dot{Q}_{sys}$ to the intestines. Absolute tissue flows were greater to muscle (4.8-fold), bone (2.6-fold), intestines (10.4-fold), liver (2.5-fold), gonads (2.4-fold) and fat (2.5-fold) in warm turtles.

Systemic blood flow distribution was altered with anoxic submergence. After 6 h of anoxia at 21°C, $\%\dot{Q}_{sys}$ decreased significantly in the stomach (6.2-fold) and intestines (3.8-fold), and increased significantly in the muscle (1.3-fold) and shell (1.7-fold) (Fig. 4C,D; Table 5). Absolute tissue blood flow decreased significantly to the intestines (14.4-fold), stomach (11.8-fold), kidneys (10.7-fold) and muscle (1.9-fold) (Table 5). After a 12-day anoxic exposure at 5°C, $\%\dot{Q}_{sys}$ decreased significantly in the kidneys (2.7-fold) and gonads (2.2-fold) and increased significantly in the liver (1.7-fold) and shell (1.2-fold) (Fig. 4A,B; Table 5). Absolute blood flow decreased to all systemic tissues during anoxic submergence at 5°C (Table 5), with the largest decreases

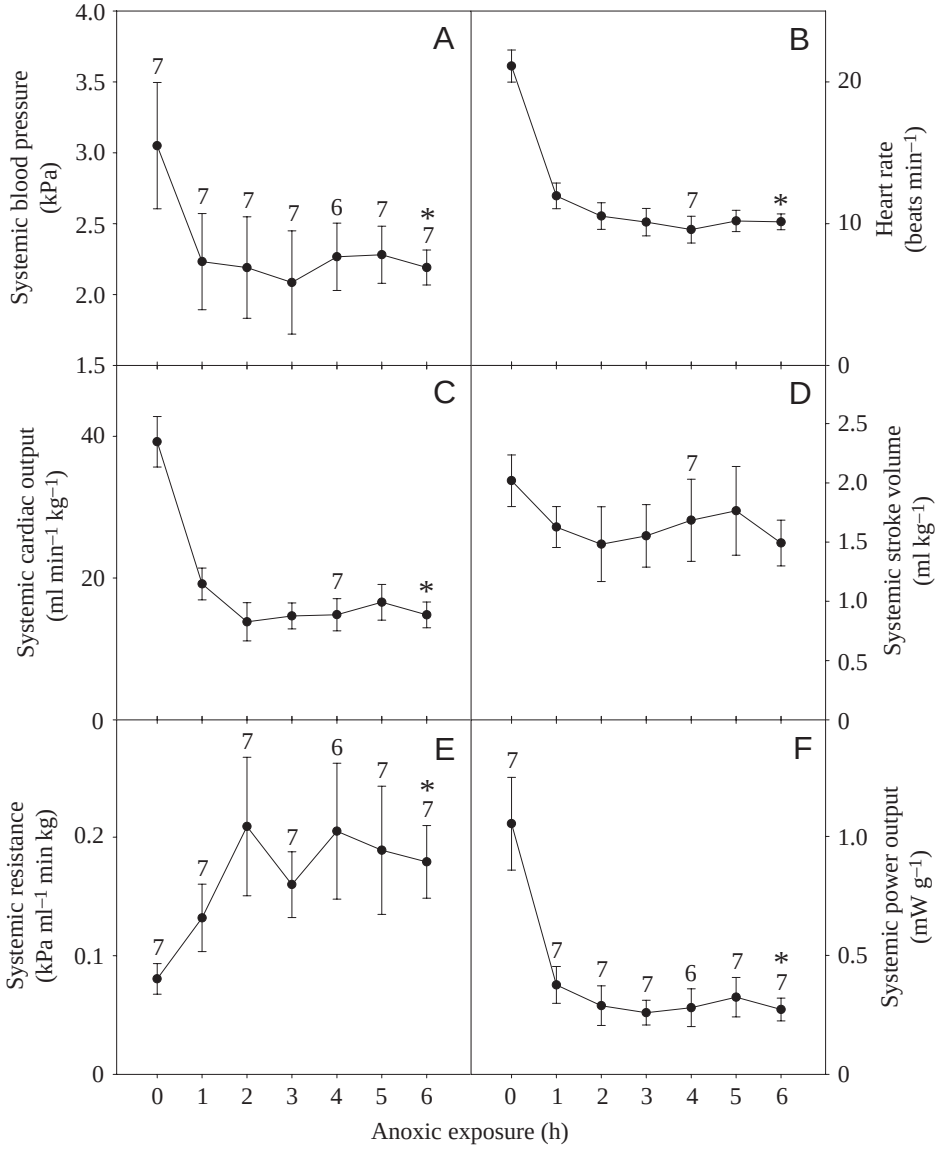

Fig. 2. Chronological changes of cardiovascular function in 21°C-acclimated turtles during 6 h of anoxic submergence. Asterisks indicate significant differences (P<0.05) of each final anoxic measurement from normoxic control (time zero). Values are means ± S.E.M.; N=8 unless otherwise indicated above the error bar.

Table 3. *Cardiovascular status of 5°C- and 21°C-acclimated normoxic turtles before and after intra-arterial injection of the α-adrenergic antagonist phentolamine*

Acclimation temperature	Drug injected	Heart rate f_H (beats min^{-1})	Systemic stroke volume V_{Ssys} (ml kg^{-1})	Systemic cardiac output $\dot{Q}_{sys}$ (ml min^{-1} kg^{-1})	Systemic blood pressure P_{sys} (kPa)	Systemic power output PO_{sys} (mW g^{-1})	Systemic resistance R_{sys} (kPa ml^{-1} min kg)
5°C	Routine normoxic	4.1±0.4*	2.31±0.37	9.2±1.3*	1.52±0.11*	0.126±0.025*	0.18±0.03*
	Phentolamine (3 mg kg^{-1})	4.3±0.5	3.21±0.57†	12.8±1.8†	0.98±0.06†	0.108±0.015	0.08±0.01†
21°C	Routine normoxic	21.3±2.0	1.95±0.29 (5)	42.9±8.0 (5)	2.83±0.53	1.508±0.323 (5)	0.09±0.02 (5)
	Phentolamine (3 mg kg^{-1})	22.6±3.4	2.84±0.90 (4)	63.5±18.6 (4)	2.08±0.33	1.777±0.591 (4)	0.06±0.02 (4)

Values are means ± S.E.M. (N=6, unless otherwise indicated in parentheses).
*Significant differences (P<0.05) between acclimation temperature for routine normoxic cardiovascular status.
†Significant differences (P<0.05) between routine normoxic and phentolamine for each variable at each acclimation temperature.

Table 4. *Cardiovascular status of 5°C- and 21°C-acclimated anoxic turtles before and after intra-arterial injections of the α-adrenergic agonist phenylephrine and antagonist phentolamine during anoxic exposure*

Acclimation temperature	Drug injected	Heart rate f_H (beats min^{-1})	Systemic stroke volume V_{Ssys} (ml kg^{-1})	Systemic cardiac output $\dot{Q}_{sys}$ (ml min^{-1} kg^{-1})	Systemic blood pressure P_{sys} (kPa)	Systemic power output PO_{sys} (mW g^{-1})	Systemic resistance R_{sys} (kPa ml^{-1} min kg)
5°C	Routine normoxic	4.96±0.38	2.79±0.51	13.62±2.34	1.81±0.16	0.228±0.052	0.14±0.01
	Routine anoxic	1.06±0.11^{a}	3.10±0.40	3.18±0.33	1.22±0.14	0.034±0.006	0.41±0.08
	Phenylephrine (5 μg kg^{-1})	1.13±0.06^{a}	2.42±0.33^{a}	2.71±0.36	1.75±0.18^{a}	0.041±0.007	0.74±0.16^{a}
	Phenylephrine (50 μg kg^{-1})	1.33±0.07^{b}	2.03±0.42^{a}	2.64±0.62	2.11±0.18^{b}	0.045±0.009	1.02±0.19^{b}
	Phentolamine (3 mg kg^{-1})	1.25±0.08^{a,b}	3.58±0.69	4.70±1.12^{a}	1.36±0.13	0.055±0.015	0.46±0.20
	Phenylephrine (50 μg kg^{-1})	1.20±0.07^{a,b}	3.84±0.75	4.67±1.07^{a}	1.37±0.10	0.056±0.014	0.40±0.13
21°C	Routine normoxic	21.1±1.1 (8)	2.02±0.22 (8)	39.2±3.6 (8)	3.05±0.45 (7)	1.053±0.195 (7)	0.08±0.01 (7)
	Routine anoxic	10.1±0.6 (8)	1.49±0.19 (8)	14.8±1.8 (8)	2.19±0.12 (7)^{a}	0.272±0.048 (7)	0.18±0.03 (7)
	Phenylephrine (5 μg kg^{-1})	10.4±0.5 (8)	1.39±0.23 (8)	14.3±2.4 (8)	2.71±0.18 (7)^{b}	0.313±0.056 (7)	0.27±0.07 (7)
	Phenylephrine (50 μg kg^{-1})	10.5±0.4 (8)	1.45±0.25 (8)	15.0±2.3 (8)	2.41±0.19 (7)^{a,b}	0.300±0.060 (7)	0.21±0.04 (7)
	Phentolamine (3 mg kg^{-1})	11.9±0.5 (8)^{a}	2.07±0.20 (8)^{a}	24.4±2.5 (8)^{a}	1.24±0.13 (7)	0.252±0.037 (7)	0.06±0.01 (7)^{a}
	Phenylephrine (50 μg kg^{-1})	12.4±0.7 (8)^{a}	1.96±0.20 (8)^{a}	23.9±2.3 (8)^{a}	1.41±0.16 (7)	0.288±0.056 (7)	0.07±0.01 (7)^{a}

Values are means ± S.E.M. (N=6 at 5°C and are indicated in parentheses at 21°C).
Significant differences (P<0.05) between routine anoxic and drug injections for each variable at each acclimation temperature are indicated by dissimilar letters.

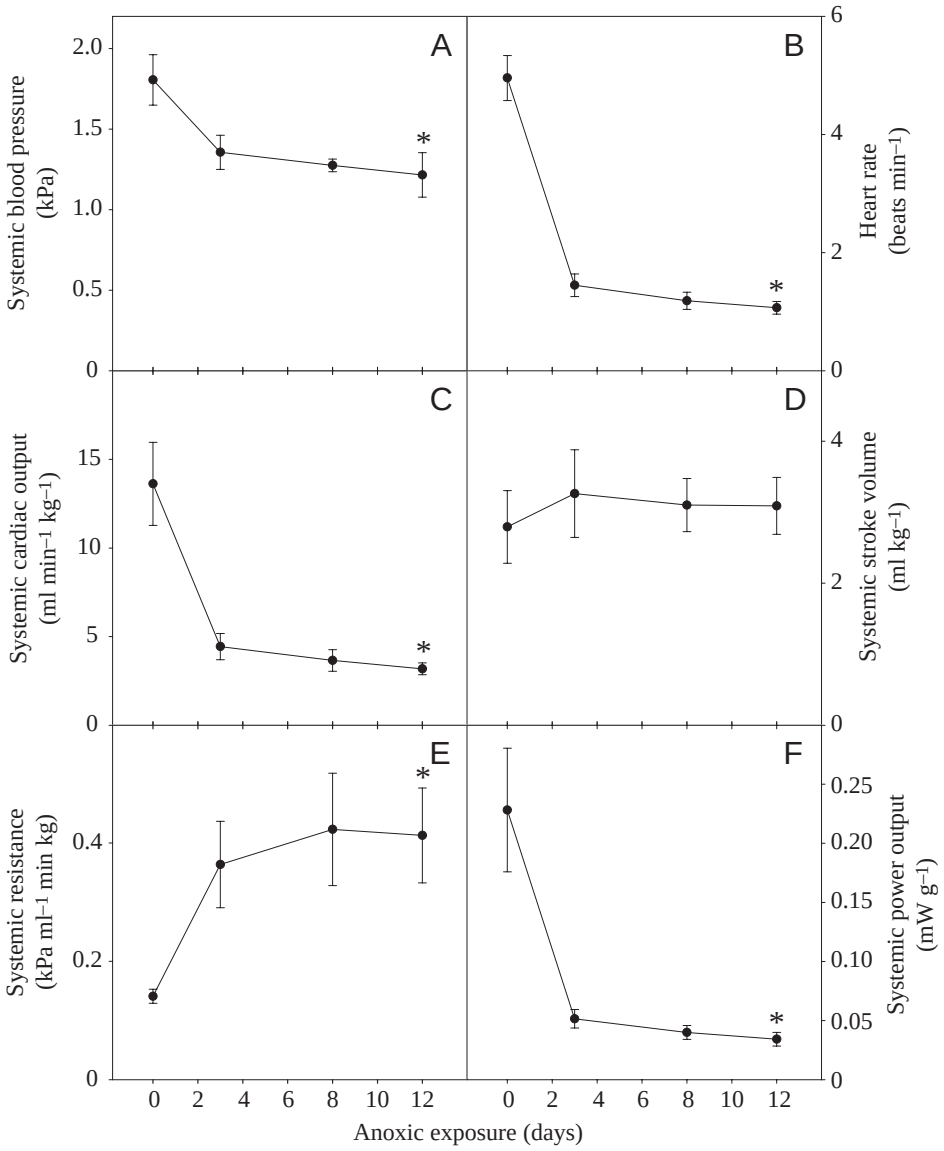

Fig. 3. Chronological changes of cardiovascular function in 5°C-acclimated turtles during 12 days of anoxic submergence. Asterisks indicate significant differences (P<0.05) of each final anoxic measurement from normoxic control (time zero). Values are means ± S.E.M.; N=6.

occurring in the digestive and urogenital tissues and the smallest decreases occurring in the brain, heart and liver (Table 6).

α-Adrenergic control of cardiovascular function and systemic blood flow distribution during anoxia

Systemic α-adrenergic tone in anoxic turtles differed with acclimation temperature, but in contrast to normoxic turtles, systemic α-adrenergic tone increased with acclimation temperature. With the exception of a significant, but minor increase in P_{sys} after a low dose of phenylephrine (5 μg kg^{-1}), there were no significant effects of α-adrenergic stimulation at 21°C, although R_{sys} did tend to increase (Table 4). In contrast to these small effects of α-adrenergic stimulation, injection of the α-adrenergic antagonist phentolamine elicited a threefold decrease in R_{sys} at 21°C and completely abolished the effects of subsequent injection of phenylephrine. Thus, the large anoxia-induced increase in R_{sys} at 21°C was a result of an elevated α-adrenergic tone.

In anoxic turtles at 5°C, there was a clear dose-dependent increase in P_{sys} and R_{sys} following injection of phenylephrine, but phentolamine did not significantly affect routine anoxic R_{sys} (Table 4). Thus, although α-adrenergic receptors remained functional, as attested by the eliminated effects of phenylephrine following phentolamine (Table 4), the increase in R_{sys} during anoxia was not a result of an increased α-adrenergic tone. Consequently, the anoxia-induced α-adrenergic mediated systemic vasoconstriction was blunted at 5°C despite being central to the systemic vascular tone during normoxia at this temperature.

Due to large individual variation there were no significant changes in either relative or absolute tissue blood flows following α-adrenergic manipulation in anoxic turtles at 21°C (Table 5). At 5°C, phenylephrine injection significantly increased $\%\dot{Q}_{sys}$ in muscle and liver, while $\%\dot{Q}_{sys}$ to the shell decreased. Similarly, absolute blood flow to the liver increased following α-adrenergic stimulation. However, only the increased liver $\%\dot{Q}_{sys}$, which occurred with α-stimulation, was restored to routine anoxic levels with phentolamine injection. Shell $\%\dot{Q}_{sys}$ increased significantly following α-adrenergic blockade, but did not fully return to the pre-α-adrenergic stimulation value.

Discussion

Normoxic cardiovascular function and systemic blood flow distribution: effects of temperature

Turtles were allowed to recover for 48–72 h after surgery to reduce the effects of surgical stress. Control normoxic hematocrit and arterial pH, recorded prior to anoxic exposure, are within previously reported ranges (Jackson and Ultsch, 1982; Ultsch and Jackson, 1982; Hicks and Farrell, 2000b) and indicate a successful post-operative recovery. Additionally, normoxic $\dot{Q}_{sys}$, P_{sys}, PO_{sys} and R_{sys} at 21°C are similar to those

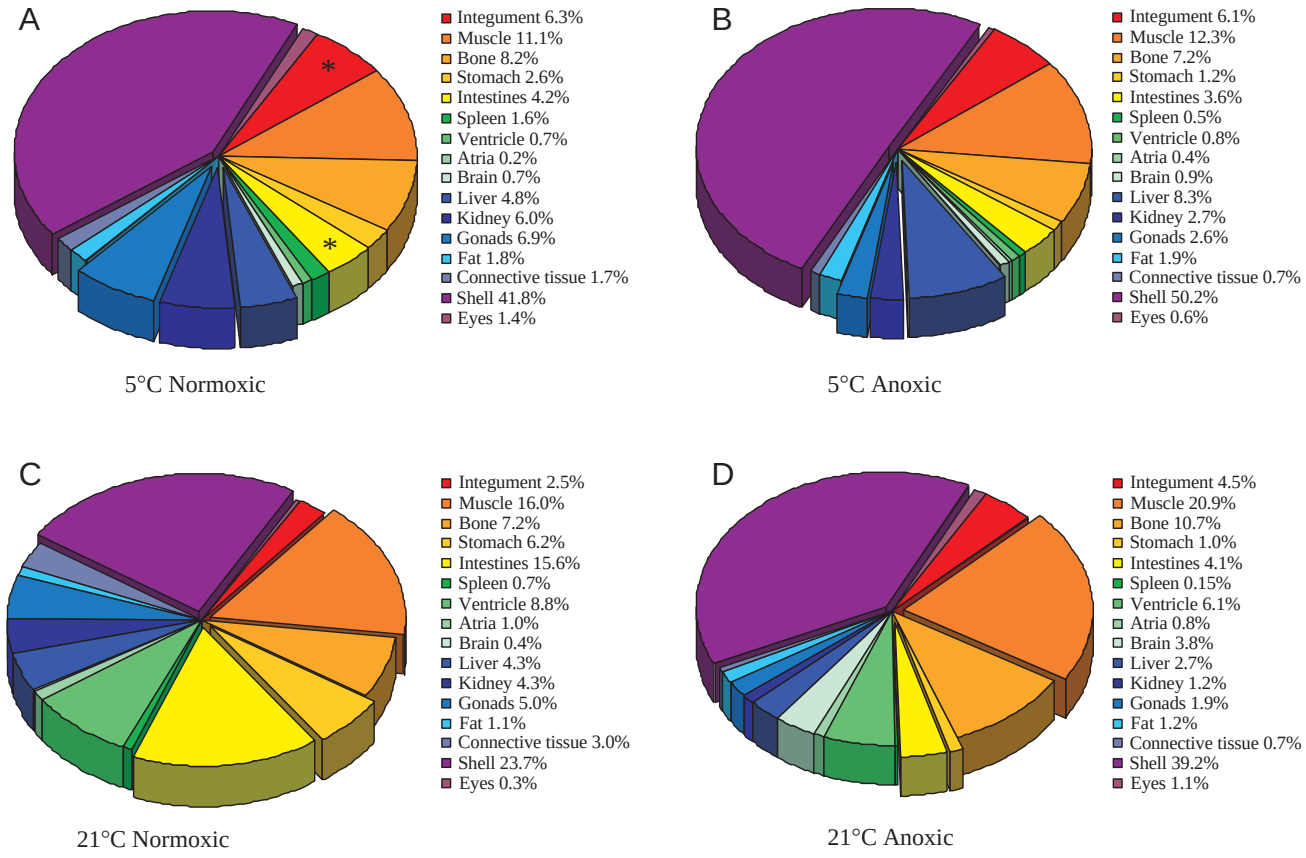

Fig. 4. Percent of systemic cardiac output (%$\dot{Q}_{sys}$) distributed between tissues from turtles acclimated at 5°C (A,B) and 21°C (C,D). (A) Routine normoxic control at 5°C, (B) after 12 days of anoxic submergence at 5°C, (C) routine normoxic control at 21°C and (D) after 6 h of anoxic submergence at 21°C. Asterisks indicate significant (P<0.05) tissue specific differences in %$\dot{Q}_{sys}$ between acclimation temperatures in routine normoxic control. Exploded slices signify significant differences (P<0.05) in tissue %$\dot{Q}_{sys}$ between routine normoxia and anoxia within each acclimation temperature. N=6 at 5°C and 5 at 21°C.

reported in previous studies on turtles at 20–25°C (Shelton and Burggren, 1976; Herbert and Jackson, 1985b; Hicks, 1994; Wang and Hicks, 1996; Hicks and Wang, 1998; Hicks and Farrell, 2000a,b). At 5°C, our values for f_H and P_{sys} during normoxia are also similar to previously reported values (Herbert and Jackson, 1985b; Hicks and Farrell, 2000a,b), but our $\dot{Q}_{sys}$ at 5°C (9–14 ml min^{-1} kg^{-1}) is greater than the value of 4.0 ml min^{-1} kg^{-1} reported by Hicks and Farrell (2000a,b). Consequently, normoxic VS_{sys} and PO_{sys} at 5°C are approximately fourfold higher and R_{sys} twofold lower than reported by Hicks and Farrell (2000a,b). These quantitative differences may reflect the different 5°C acclimation procedures used in the two studies, or seasonal variability in the response to anoxia; our study was performed in winter/spring, while the study by Hicks and Farrell (2000a,b) was performed in the fall/winter.

The reduction in $\dot{Q}_{sys}$ with reduced temperature during normoxia is consistent with other measurements from ectothermic vertebrates, including freshwater turtles (Farrell and Jones, 1992; Hicks and Farrell, 2000a,b; Stecyk and Farrell, 2002; J. A. W. Stecyk and A. P. Farrell, manuscript submitted for publication), and mirrors the reduction in metabolic rate (Jackson and Schmidt-Nielsen, 1966; Jackson, 1968; Herbert and Jackson, 1985b). Although P_{sys} was also reduced with decreased temperature, R_{sys} was greatly elevated at 5°C. α-Adrenergic regulation of systemic vasomotor tone seems important in this response since α-adrenergic blockade with phentolamine injection at 5°C reduced R_{sys} to the 21°C normoxic level, but was without effect on R_{sys} at 21°C (Tables 3, 4). The increased α-adrenergic vasomotor tone in 5°C normoxic turtles supplements the suppression of cholinergic inhibition of the heart at low temperature, which probably offsets the negative effects of temperature on cardiac activity (Hicks and Farrell, 2000b), and may represent an important mechanism for regulating blood flow distribution between priority and less-essential tissues. In fact, differences in absolute blood flow and %$\dot{Q}_{sys}$ existed between warm- and cold-acclimated turtles. Absolute blood flow was decreased to muscle, bone, intestines, liver, gonads and fat in 5°C acclimated turtles relative to 21°C acclimated turtles. Additionally, %$\dot{Q}_{sys}$ was increased to the integument at 5°C, which may reflect the increased reliance on cutaneous gas exchange at this temperature (Herbert and Jackson, 1985b; Ultsch and Jackson, 1982). The decrease in %$\dot{Q}_{sys}$ to the

Table 5. *Percent systemic cardiac output (%$\dot{Q}_{sys}$) and absolute blood flow (μl min^{-1} kg^{-1}) to the various tissues of 5°C- and 21°C-acclimated turtles during routine normoxic and anoxic conditions and following anoxic injections of phenylephrine and phentolamine*

			%$\dot{Q}_{sys}$				Tissue flow (μl min^{-1} g^{-1})			
Acclimation temperature	Tissue	Relative mass (%)	Routine normoxia	Routine anoxia	Phenylephrine (50 μg kg^{-1})	Phentolamine (3 mg kg^{-1})	Routine normoxia	Routine anoxia	Phenylephrine (50 μg kg^{-1})	Phentolamine (3 mg kg^{-1})
5°C	Integument	4.4±0.2	6.3±1.3‡	6.1±1.1	3.4±0.4	5.3±0.8	20.9±4.9[a]	5.0±1.0	2.6±0.5	6.6±1.9
	Muscle	19.7±0.9	11.1±1.5	12.3±1.4[a]	17.0±2.9[b]	19.1±1.8[b]	8.7±1.7‡[a]	2.4±0.4	2.9±0.6	5.5±1.4
	Bone	6.6±0.6	8.2±0.9	7.2±1.0	5.5±1.1	9.1±0.7	23.0±5.1‡[a]	4.7±1.0	3.3±0.8	8.8±2.0
	Stomach	2.3±0.4	2.6±1.3	1.2±0.3	0.8±0.2	1.0±0.4	31.8±18.1[a]	2.4±0.5	1.6±0.4	2.2±0.7
	Intestines	4.8±0.8	4.2±0.8‡	3.6±1.5	3.0±1.0	4.1±1.6	17.8±4.8‡[a]	3.3±1.3	2.5±0.8	4.9±1.7
	Spleen	0.3±0.1	1.6±0.9	0.5±0.1	0.6±0.4	0.5±0.2	100.0±50.78[a]	9.8±3.2	8.0±3.9	13.9±5.9
	Ventricle	0.2±0.02	0.7±0.3	0.8±0.3	0.6±0.2	0.8±0.4	38.4±15.5[a]	11.1±4.1	8.2±3.2	13.3±5.9
	Atria	0.07±0.02	0.2±0.05	0.4±0.1	0.5±0.08	0.5±0.1	40.1±9.1[a]	17.4±4.2	19.7±5.2	22.8±4.2
	Brain	0.3±0.2	0.7±0.3	0.9±0.4	0.3±0.09	0.3±0.2	121.4±43.3[a]	38.1±16.8	13.2±4.6	15.2±11.3
	Liver	9.4±0.6	4.8±1.1	8.3±2.1*[a]	27.3±6.7[b]	9.1±1.4[a]	9.3±2.2‡[a]	3.7±0.9[b]	12.8±5.5[a]	5.8±1.3[a,b]
	Kidneys	0.9±0.3	6.0±3.2	2.7±1.7*[b]	3.2±2.1	1.0±0.2	223.7±160.1‡[a]	15.8±8.4	16.8±9.9	9.3±3.5
	Gonads	8.7±2.6	6.9±2.5	2.6±0.4*	3.4±1.6	3.2±0.7	16.5±4.9‡[a]	3.2±1.6	2.5±1.2	6.3±3.5
	Fat	2.9±0.6	1.8±0.4	1.9±0.7	1.9±0.8	2.3±0.7	7.3±1.5‡[a]	1.8±0.4	2.0±0.9	3.5±1.3
	Connective tissue	1.9±0.2	1.7±0.4	0.7±0.2	0.8±0.2	1.3±0.3	21.9±4.1[a]	2.6±0.7	2.5±0.5	5.9±1.5
	Shell	35.2±2.1	41.8±7.5	50.2±4.9*[a]	31.3±5.5[b]	42.5±3.0[c]	19.6±6.5[a]	5.2±0.9	3.0±0.9	6.4±1.7
	Eyes	0.07±0.02	1.4±0.5	0.6±0.3	0.2±0.1	0.1±0.02	347.8±118.0[a]	41.4±17.8	13.5±6.4	12.6±3.0
21°C	Integument	4.5±0.1	2.5±0.4	4.5±1.0	5.4±0.8	4.3±0.6	32.1±7.2	20.4±7.1	20.4±3.7	26.6±5.5
	Muscle	20.2±0.5	16.0±3.8	20.9±7.0*	16.6±3.3	22.5±6.0	41.0±11.6[a]	21.7±8.7[b]	16.0±4.9[b]	33.5±12.3[a,b]
	Bone	6.0±0.2	7.2±1.0	10.7±1.6	8.0±1.8	12.9±4.8	58.9±11.3[a]	28.8±5.3[a,b]	19.3±3.1[b]	45.4±12.1[a,b]
	Stomach	1.7±0.2	6.2±4.2	1.0±0.3*	3.3±1.4	0.8±0.3	84.9±31.3[a]	7.2±1.8[b]	21.0±6.3[b]	9.4±3.4[b]
	Intestines	4.0±0.4	15.6±3.5	4.1±1.4*	10.4±7.4	3.5±1.1	185.2±59.9[a]	12.9±2.9	31.4±19.3	17.0±2.5
	Spleen	0.2±0.02	0.7±0.3	0.2±0.1	0.6±0.3	0.3±0.1	217.5±116.5	8.5±4.0	41.3±30.7	24.0±11.3
	Ventricle	0.2±0.01	8.8±5.4	6.1±3.1	1.6±0.7	3.9±2.6	2354.4±1653.6	326.2±125.0	89.7±27.9	294.3±151.1
	Atria	0.1±0.07	1.0±0.6	0.8±0.5	0.3±0.09	0.4±0.1	2250.5±1798.2	491.4±438.4	110.4±48.7	270.9±197.5
	Brain	0.08±0.01	0.4±0.2	3.8±1.9	0.6±0.7	0.5±0.5	145.3±71.5	480.1±305.5	95.8±81.2	3.6±21.2
	Liver	8.4±1.0	4.3±0.8	2.7±0.9	9.5±5.1	4.9±1.8	23.7±4.3	5.3±2.1	22.0±15.4	14.9±6.8
	Kidneys	0.6±0.05	4.3±2.5	1.2±0.5	0.4±0.2	0.8±0.2	317.9±176.7[a]	29.8±14.3	10.1±4.7	28.7±9.6
	Gonads	8.5±2.3	5.0±1.9	1.9±0.8	4.6±1.9	2.8±0.9	39.5±9.6	42.6±39.3	48.5±38.8	59.6±5.14
	Fat	3.7±0.8	1.1±0.3	1.2±0.3	1.9±0.7	1.9±0.4	18.4±4.3	8.3±2.8	11.1±4.0	17.9±4.0
	Connective tissue	1.2±0.2	3.0±1.9	0.7±0.1	1.0±0.1	1.4±0.2	77.9±48.3	7.2±1.9	10.7±3.1	23.3±7.0
	Shell	38.0±1.2	23.7±6.7	39.2±5.2*	35.9±7.9	39.2±7.3	35.5±11.4	19.9±4.2	17.5±4.0	29.7±6.5
	Eyes	0.06±0.01	0.3±0.1	1.1±0.6	0.1±0.06	0.06±0.04	497.0±236.8	1450.5±1296.2	80.4±52.1	39.1±2.14

Values are means ± S.E.M. (N=6 at 5°C; N=5 at 21°C).
*Significant differences (P<0.05) in %$\dot{Q}_{sys}$ for each tissue between routine normoxia and routine anoxia.
‡Significant differences (P<0.05) in normoxic %$\dot{Q}_{sys}$ and absolute blood flow between acclimation temperature.
Significant differences (P<0.05) in %$\dot{Q}_{sys}$ for each tissue between routine anoxia, phenylephrine and phentolamine injections are indicated with dissimilar letters.
Significant differences (P<0.05) in absolute blood flow for each tissue between the four conditions are indicated by dissimilar letters.

Table 6. *Reductions in tissue absolute flow after 12 days of anoxic submergence at 5°C*

Tissue	Fold change
Kidneys	14.2
Stomach	13.3
Spleen	10.2
Connective tissue	8.4
Eyes	8.4
Intestines	5.4
Gonads	5.2
Bone	4.9
Integument	4.2
Fat	4.1
Shell	3.8
Muscle	3.6
Ventricle	3.5
Brain	3.2
Liver	2.5
Atria	2.3

intestines at 5°C compared to 21°C may reflect the fact that these animals had fasted during the 1.5-month acclimation period. However, verification of an α-adrenergic involvement in these phenomena is still needed.

Control of systemic peripheral resistance during anoxia

As previously reported, anoxia was accompanied by large depressions in f_H, $\dot{Q}_{sys}$ and P_{sys} at both 5°C and 21°C (Figs 2, 3; Table 4). These cardiovascular changes closely resemble those previously described at warm and cold temperatures (Herbert and Jackson, 1985b; Hicks and Wang, 1998; Hicks and Farrell, 2000a), although our $\dot{Q}_{sys}$ value was higher than that reported by Hicks and Farrell (2000a,b) due to an elevated V_{Ssys}. The marked increase in R_{sys} accompanying anoxia at both temperatures is also consistent with earlier studies (Hicks and Wang, 1998; Hicks and Farrell, 2000a,b), but contrasts with the normal vasodilatory effects of oxygen lack, decreased pH and increased levels of vasoactive metabolites that are present during anoxia. Thus, the increased R_{sys} may be due to activation of vascular α-adrenergic receptors by the elevated levels of circulating catecholamines (Wasser and Jackson, 1991; Keiver and Hochachka, 1991; Keiver et al., 1992) and/or increased sympathetic nerve activity. Indeed, α-adrenergic peripheral vasoconstriction during oxygen limitation is well documented in different vertebrates (Butler and Jones, 1971; Lillo, 1979; Butler, 1982; Fritsche and Nilsson, 1989; Axelsson and Fritsche, 1991; Lacombe and Jones, 1991; Signore and Jones, 1995; J. A. W. Stecyk and A. P. Farrell, manuscript submitted for publication), and *Trachemys scripta* certainly has α-adrenergic receptor mediated control of R_{sys} (e.g. Overgaard et al., 2002). However, autonomic regulation of cardiac activity during anoxia in turtles is dependent upon acclimation temperature. Autonomic control is more pronounced at warm acclimation temperatures, while the direct effects of oxygen lack and acidosis seem to account for most of the decreased cardiac performance during cold, anoxic submergence (Hicks and Wang, 1998; Hicks and Farrell, 2000b).

The present study reveals that the α-adrenergic system remains functional during anoxia at both warm and cold acclimation temperatures, but that the α-adrenergic contribution to the increased R_{sys} varies with temperature. Specifically, a large α-adrenergic tone accounting for the increased R_{sys} during anoxia at 21°C was revealed by the lack of haemodynamic responses following injection of phenylephrine and the large (threefold) reduction in R_{sys} following α-adrenergic blockade with phentolamine. In fact, phentolamine reduced R_{sys} to the 21°C normoxic level (Tables 3, 4). The small effect of phenylephrine injection on cardiovascular function may possibly reflect the high levels of circulating catecholamines (>56 nmol norepinephrine; Wasser and Jackson, 1991) fully saturating the systemic α-adrenergic receptors during anoxia.

In contrast to the high α-adrenergic tone on R_{sys} during anoxia at 21°C, the large progressive increase in R_{sys} accompanying anoxia at 5°C does not seem to be mediated by α-adrenergic vasoactivity. While inhibition of the α-adrenergic receptors with phentolamine eliminated the effects of the preceding α-adrenergic stimulation with phenylephrine, α-adrenergic blockade did not affect routine anoxic R_{sys} at 5°C. This finding is peculiar because it demonstrates that α-adrenergic vasoactivity remains operational during anoxia, but that the systemic α-adrenergic tonus is low. Thus, the increased concentration of plasma catecholamines present in cold, anoxic turtles (approximately 25 nmol norepinephrine; Wasser and Jackson, 1991) seemingly does not elicit α-adrenergic mediated systemic vasoconstriction, perhaps because of increased receptor density, decreased receptor affinity, or reduced signal transduction efficacy. Nevertheless, the low α-adrenergic tone during anoxia at 5°C is consistent with an overall blunting of the autonomic regulation of the cardiovascular system during cold anoxic submergence, when only small cholinergic and β-adrenergic tones exist on the cardiovascular system (Hicks and Farrell, 2000b).

Given the low α-adrenergic tone on the systemic circulation during anoxia at 5°C, other regulatory mechanisms must be responsible for the increased R_{sys}. Hicks and Farrell (2000a) suggested that the hypotension associated with anoxia at 5°C could directly affect R_{sys} if P_{sys} failed to surpass the critical closing pressure of certain vessels. However, our results from normoxic turtles at 5°C argue against such a mechanism because injection of phentolamine caused a very severe hypotension (<1.0 kPa) while R_{sys} remained low (Table 3). Nonetheless, vessel diameters may be reduced at low blood flows and pressures, leading to a higher resistance (Lipowsky et al., 1978). Similarly, blood vessel tension is increased with cold temperature, and thus may also contribute to the increased R_{sys} (Friedman et al., 1968; Dinnar, 1981), which would be exacerbated by the increased blood viscosity as temperature and flow decrease (Langille and Crisp, 1980). Finally, the low α-adrenergic vasomotor tone during anoxia may represent increased non-adrenergic, non-cholinergic regulation of R_{sys}.

Changes in systemic blood flow distribution with anoxic exposure

A redistribution of blood flow towards oxygen-sensitive tissues such as the brain and heart is critical to survival and is a commonly used survival strategy among vertebrates when exposed to hypoxia (Johansen, 1964; Elsner et al., 1966; Chalmers et al., 1967; Krasney, 1971; Butler and Jones, 1971; Jones et al., 1979; Zapol et al., 1979; Boutilier et al., 1986; Davies, 1989, 1991; Bickler, 1992; Hylland et al., 1994, 1996; Nilsson et al., 1994; Yoshikawa et al., 1995; Söderström et al., 1999). Here, we provide a quantitative description of systemic blood flow distribution during the large depression in cardiac status occurring with anoxic submergence in the anoxia-tolerant freshwater turtle. We clearly show that perfusion is sacrificed in subsidiary tissues, while the cerebral and myocardial circulations, as well as other critical organs, receive a priority of blood flow. Specifically, anoxia led to substantial depressions in $\%\dot{Q}_{sys}$ and absolute tissue flow to digestive and urogenital organs at both temperatures, while $\%\dot{Q}_{sys}$ was increased or maintained to the shell (5°C and 21°C), muscle (21°C) and liver (5°C) (Fig. 4, Table 5). These findings are in agreement with the redistribution of blood flow away from the renal and splanchnic circulatory beds observed after 30 min of N_2 ventilation in anaesthetized turtles (Davies, 1989) and are consistent with the greatly reduced renal function in conscious anoxic turtles (Warburton and Jackson, 1991; Jackson et al., 1996). Furthermore, the small number of microspheres directed towards the lungs during anoxia at 5°C implies the presence of a similar pulmonary vasoconstriction and reduced Left-to-Right shunt with anoxic exposure at 5°C to that exhibited during anoxic submergence at warm temperatures (Hicks and Wang, 1998; Crossley et al., 1998).

The importance of tissues containing large glycogen stores, specifically the liver and skeletal muscle, in fostering anoxic survival is highlighted in the present study. During anoxia, turtles must meet their energy demands through anaerobic metabolism, with glucose as the primary substrate. Glucose is derived from catecholamine-mediated breakdown of hepatic and skeletal muscle glycogen stores (Daw et al., 1967; Penny, 1974; Keiver and Hochachka, 1991; Wasser and Jackson, 1991; Keiver et al., 1992), thus, maintained blood flow to the liver and muscle during anoxia may facilitate glucose export to other organs. Indeed, 6 h of anoxia at 21°C resulted in an increased $\%\dot{Q}_{sys}$ to muscle and the maintenance of liver absolute blood flow at control normoxic levels. Similarly, $\%\dot{Q}_{sys}$ to the liver was increased during anoxia at 5°C, and the reduction in absolute flows to the liver and muscle were minimal compared with the overall reduction in $\dot{Q}_{sys}$ (4.3-fold, Table 4) and the reductions in absolute blood flow to the bulk of systemic tissues (Table 6).

Anaerobic energy metabolism potentially threatens anoxic survival because of the accumulation of lactate and the ensuing acidosis (Herbert and Jackson, 1985b). However, the shell of the turtle acts as a powerful buffer reserve and diminishes the acidosis and accumulation of lactate in body fluids (reviewed by Jackson, 2000, 2002). The increased demand of blood flow to the shell observed in the present study is consistent with the increased demand on the shell as a buffer reserve during anoxia. In fact, $\%\dot{Q}_{sys}$ directed to the shell increased significantly during anoxia at both temperatures, such that after cardiac depression, 40–50% of $\dot{Q}_{sys}$ was directed towards the shell. Furthermore, the homogenous distribution of microspheres in the shell is consistent with the uniform lactate accumulation of the entire shell during anoxia (Jackson et al., 1996; Jackson, 1997).

Turtles in the present study did not display the increased $\%\dot{Q}_{sys}$ or absolute blood flow to the brain or myocardial circulations documented during short-term hypoxic exposure in other vertebrates (Johansen, 1964; Elsner et al., 1966; Chalmers et al., 1967; Krasney, 1971; Butler and Jones, 1971; Jones et al., 1979; Zapol et al., 1979; Nilsson et al., 1994; Yoshikawa et al., 1995; Söderström et al., 1999), including anaesthetized freshwater turtles (Davies, 1989, 1991; Bickler, 1992; Hylland et al., 1994, 1996). Nevertheless, the importance of the brain and heart for anoxic survival and their corresponding demand for blood flow is clearly signified in the present study. At 21°C, brain and myocardial $\%\dot{Q}_{sys}$ and absolute blood flow were maintained at control normoxic levels following the 6 h exposure period despite a 2.6-fold decrease in $\dot{Q}_{sys}$ (Fig. 2, Tables 4, 5). Likewise, 5°C cerebral and myocardial $\%\dot{Q}_{sys}$ were maintained at control normoxic levels after 12 days of anoxia, while absolute blood flows were reduced less than the overall reduction in $\dot{Q}_{sys}$ (4.3-fold; Table 4), as well as the reductions in absolute blood flows to the bulk of the systemic tissues (Table 6). These differences in cerebral and myocardial blood supply during anoxia may simply reflect the long duration of anoxia in our study, which resulted in a complete transition from aerobic to anaerobic metabolism. Typically, normal cellular functions are maintained at the onset of anoxia and organ ATP levels preserved through activation of glycolysis. Consequently, increased tissue blood flow may be required for increased glucose delivery and waste removal. However, once biochemical reorganization has occurred, and glycolytic inhibition (reviewed by Storey, 1996) and metabolic depression are established, an increase in blood flow is no longer required. In fact, at 20°C, brain blood flow of anaesthetized anoxic turtles returns to normoxic levels within 1–2 h of anoxia (Hylland et al., 1994, 1996).

α-Adrenergic control of systemic blood flow distribution during anoxia

It is well established that α-adrenergic control mediates peripheral vasoconstriction and subsequent redistribution of blood flow among tissues during hypoxia or diving in many groups of vertebrates (Butler and Jones, 1971; Butler, 1982; Lacombe and Jones, 1991; Signore and Jones, 1995). In the present study, the use of microspheres was unable to resolve many major changes in blood flow distribution between tissues following injection of α-adrenergic agonists and antagonists, and this, to some extent, may reflect a limitation of the

methodology. However, given that α-adrenergic stimulation did not increase R_{sys} during anoxia at 21°C, and given that R_{sys} is not α-adrenergically mediated during anoxia at 5°C, major changes in blood flow distribution may not be expected after α-adrenergic manipulation. Nevertheless, there seems to be an α-adrenergic mediated dilation of the liver and constriction in the shell during anoxia at 5°C (Table 5). Conversely, the general lack of changes at 21°C may simply reflect a global response of all tissues to α-adrenergic manipulation, with the resistances in all tissue beds changing simultaneously such that no overall redistribution occurs. Differentiation between the two possibilities is outside the scope of the present study and thus caution must be exercised in interpreting the observed changes in $\%\dot{Q}_{sys}$ and absolute blood flow as a reflection of tissue-specific α-adrenergic regulation.

Concluding remarks

In summary, our study reveals that α-adrenergic regulation of R_{sys} in the freshwater turtle during anoxic submergence is temperature-dependent. The increased R_{sys} during anoxia at 21°C can largely be ascribed to an increased α-adrenergic tone, whereas an α-adrenergic tone does not seem to contribute to the marked increase in R_{sys} accompanying anoxia at 5°C. The large α-adrenergic tone on R_{sys} during anoxia at 21°C is consistent with the importance of autonomic regulation of the cardiovascular system during anoxia at warm temperatures, and the blunting of this response with cold anoxic exposure is consistent with the suppression of autonomic control during cold anoxic submergence. However, while the intrinsic effects of anoxia and acidosis are predominantly responsible for the depression in cardiac activity during anoxic submergence at 5°C, the primary determinants of the increased R_{sys} and regulated hypotension remain to be identified.

The overall redistribution of systemic blood flow and changes in absolute blood flows to specific tissues during anoxia are consistent with tissue metabolism and/or their respective importance for survival during anoxia. Following 6 h of anoxia at 21°C, $\%\dot{Q}_{sys}$ and absolute blood flow were reduced to the digestive and urogenital tissues while $\%\dot{Q}_{sys}$ and absolute blood flows to the cerebral and myocardial circulations were maintained at control normoxic levels. Following 12 days of anoxia at 5°C, $\%\dot{Q}_{sys}$ was reduced to the urogenital tissues, but maintained at control normoxic levels in the brain and heart. This indicates that the digestive and urogenital tissues are of reduced importance, whereas the myocardial and cerebral circulations remain a priority. Similarly, the increased importance of liver and muscle glycogen stores in fueling anaerobic metabolism during anoxia was indicated by the increased $\%\dot{Q}_{sys}$ to the muscle (21°C) and liver (5°C) and minimally reduced absolute blood flow to the liver at 5°C. Finally, the crucial and increased importance of the turtle shell as a buffer reserve during anoxic submergence (Jackson, 2000, 2002) was highlighted by the increased $\%\dot{Q}_{sys}$ directed towards the shell with anoxia at both 5°C and 21°C.

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