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RESEARCH ARTICLE

Temperature-dependent regulation of blood distribution in snakes

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SUMMARY

Regional control of blood flow is often suggested as a mechanism for fine thermoregulatory adjustments in snakes. However, the flow of blood to different body regions at various temperatures has never been visualized to confirm this mechanism. We used ^{99m}technetium-labelled macroaggregated albumin (^{99m}Tc-MAA), a radioactive tracer, to follow the flow of blood through the bodies of garter snakes (*Thamnophis sirtalis*) near their thermal maxima and minima. We injected snakes with ^{99m}Tc-MAA at cold (6–8°C) and hot (27–32°C) temperatures and imaged them using a gamma scanner. At cold ambient temperatures, snakes significantly reduced the blood flow to their tails and significantly increased the blood flow to their heads. Conversely, at hot ambient temperatures, snakes significantly increased the blood flow to their tails and significantly reduced the blood flow to their heads. This confirms that snakes are able to use differential blood distribution to regulate temperature. Our images confirm that snakes use regional control of blood flow as a means of thermoregulation and that vasomotor control of vascular beds is likely to be the mechanism of control.

Key words: snake, thermoregulation, blood flow.

INTRODUCTION

Regional control of blood flow is a thermoregulatory mechanism used by several classes of animals to regulate their body temperatures in response to changes in environmental temperature (Steen and Steen, 1965; Hales, 1983; Turner and Tracy, 1983). At low environmental temperatures, ectotherms rely on behavioural and physiological thermoregulatory mechanisms to maintain functional body temperatures because they are not able to elevate their temperature solely from metabolic heat. In reptiles, thermoregulatory behaviours, such as basking and retreating to shade, are effective means for respectively warming and cooling during daily activity but require a substantial portion of the animal's time (Cowles and Bogert, 1944). Thus, behavioural thermoregulation reduces the time available for other activities, such as foraging and mating (Huey and Slatkin, 1976). Physiological mechanisms for regulating body temperature, for instance regional control of blood flow, can be employed while carrying out other activities to help offset the cost of behavioural thermoregulation.

In snakes, differences in body temperature along the length of an animal are often quite large. For example, Gregory found that garter snakes (*Thamnophis sirtalis*) can have heads that are up to 13°C warmer than their cloacas (Gregory, 1990). Presumably, such differences in temperature are due to controlled blood flow to different regions of the animals' bodies. Indeed, many studies have shown that snakes regulate their head temperatures within a much narrower range of temperatures than their bodies (Webb and Heatwole, 1971; Johnson, 1973; Gregory, 1990; Roark and Dorcas, 2000). Although circulatory control is often proposed or assumed as the mechanism for tight regulation of head temperature, blood flow to this region in changing ambient temperatures has yet to be quantified.

In addition to keeping their heads warmer than their bodies, snakes may also regulate their tail temperature. Infrared photography of *Thamnophis sauritus* revealed that their tails are significantly (up to 7°C) colder than their bodies at low environmental temperatures (Amiel and Wassersug, 2010). Plausibly, this distribution of heat could be the result of localized vasoconstriction resulting in decreased blood flow to the tail; however, blood flow was not measured in that study.

Regulating blood flow to the limbs is an established thermoregulatory mechanism in limbed reptiles. The high surface area to volume ratio of the limbs relative to the body means that blood can be heated and cooled more rapidly in the limbs (Dzialowski and O'Connor, 1999). However, the ability of snakes to control blood flow to their tails in order to regulate their body temperature remains unstudied. Presumably, snakes could restrict blood from entering the tail at low environmental temperatures in order to maintain elevated core temperatures, and increase blood flow to the tail when environmental temperatures are high in order to quickly dissipate heat.

An alternative suggestion for the temperature differentials observed between the bodies and tails of *T. sauritus* (Amiel and Wassersug, 2010) is differing thermal inertias resulting from the discrepancy in size between the snakes' bodies and tails. In the present paper, we evaluate these competing hypotheses. If the tail–body temperature differential is the result of restricted blood flow, then a mechanism for controlling blood flow needs to be identified. Vasomotor regulation of peripheral vascular beds (Lillywhite and Seymour, 1978; Gregory, 1990) and skeletal muscular control (Webb and Heatwole, 1971) have been suggested as potential mechanisms for regional blood flow control in snakes.

Amiel and Wassersug showed that there was a sharp decrease in temperature across the cloacas of *T. sauritus* and suggested this area as a potential location for such a regulatory mechanism (Amiel and Wassersug, 2010).

In our study we used ^{99m}technetium-labelled macroaggregated albumin (^{99m}Tc-MAA) to trace the flow of blood through the bodies of garter snakes (*Thamnophis sirtalis*, Linnaeus) at various temperatures. Gamma imaging allowed us to determine the patterns of blood flow in snakes encountering thermal extremes. We specifically looked at the heads and tails to determine whether regional control of blood flow was responsible for the heat distribution patterns recorded in previous studies.

MATERIALS AND METHODS

General experimental procedures

Ten garter snakes ranging from 45 to 66 cm were captured at various sites in the Greater Vancouver Regional District, British Columbia, Canada. Snakes were captured by hand and transported to The University of British Columbia (UBC) in breathable cotton bags. While at UBC, snakes were housed in glass or plastic terraria and offered water *ad libitum*. Snakes were offered food once, 2 days after capture, and again just prior to release. Snakes were held at UBC for a maximum of 3 weeks. Blood flow in the snakes was traced by injecting ^{99m}Tc-MAA (Lantheus Medical Imaging, Vancouver, BC, Canada) into a surgically implanted catheter, followed by gamma imaging. All snakes were released at their capture locations 2 days after imaging. All work was carried out under The University of British Columbia Animal Care Protocol A09-0070-A003 and the British Columbia Ministry of Environment Permit SU10-64029.

Surgical procedure

Animals were premedicated with 0.2–0.5 mg kg^{-1} body mass butorphanol (Torbugesic®; 10 mg ml^{-1}; Wyeth, St Laurent, QC, Canada) and induced and maintained using inhalant (AErrane; Baxter Corp., Mississauga, ON, Canada) anaesthetic. Snakes were secured on an operating table lying on their backs, and 0.25 mg bupivacaine (Marcaine; 0.5%; Hospira, Montreal, QC, Canada) was infiltrated under the skin at the incision site. The surgical site was cleaned with chlorhexidine (Hibitane®; Wyeth, Guelph, ON, Canada) scrub and saline.

When the animals reached a surgical plane of anaesthesia (determined by a lack of motor response to tail pinching), a 1.5–2.0 cm incision was made on the left side beside and just caudal to the heart. The muscle and subcutaneous tissues were sharply dissected just ventral to the ribs to expose the heart and blood vessels. The skin and ribs were retracted using stay sutures. The right aortic arch was located and cranial and caudal sutures (4-0 Vicryl; Ethicon Inc., Somerville, NJ, USA) were placed around the vessel. The cranial ligature was tied around the vessel and a curved microserrefine clamp was placed at the caudal end to prevent blood loss during catheterization. Using fine spring scissors, a small V-shaped incision was made in the lumen without transecting the vessel. A sterile saline-filled catheter [polyurethane 0.040×0.024 inches (0.10×0.60 cm) outer diameter × inner diameter; Strategic Applications Inc., Libertyville, IL, USA], was inserted into the lumen of the vessel (directed caudally) and secured just cranial to the bifurcation of the left and right aortic arch. Only one aortic arch was occluded so that systemic blood flow could be maintained. Once properly positioned, a small amount of blood was withdrawn into the catheter to ensure patency. This blood was flushed back and the catheter was tied into position using the pre-placed ligatures. A third additional ligature was placed around the vessel and catheter to secure the catheter in place. The distal end of the catheter was tunnelled subcutaneously using a 16 gauge needle, and exited on the animal's back. The skin was sutured closed using a horizontal mattress pattern (4-0 PDS; Johnson & Johnson, Markham, ON, Canada).

A small volume of blood was again withdrawn into the catheter through the exteriorized portion to ensure patency. The catheter was then 'locked' using 1:10 heparinized saline and a sterile plug. The catheter was taped in place at the exit point. After closure, the animals received 10 ml kg^{-1} lactated Ringer's solution and 0.15 mg kg^{-1} meloxicam (Metacam®; 0.5% injection; Boehringer Ingelheim, Burlington, ON, Canada), both administered subcutaneously. Supplemental heat was provided during induction, surgery and recovery.

Imaging

Snakes were prepared for gamma imaging in the Large Mammal Building of the Animal Care Facility at UBC. All procedures using ^{99m}Tc-MAA were carried out under UBC radioactivity licence number ZOOL 3218-15. In order to limit movement during imaging, snakes were held in clear, flexible PVC tubing [3/4 inch (1.905 cm) inside diameter, Klearon™ K010; Kuriyama, Schaumburg, IL, USA]. Each tube had a lateral cut along its entire length to allow access to the catheters. A window was also cut in each tube to provide adequate ventilation. On the first day of imaging, tubes containing the snakes were placed in a fridge until body temperatures reached 6–8°C. On the second day of imaging, tubes containing the snakes were placed on heating pads until body temperatures were 27–32°C. Body temperature readings were taken with a digital thermocouple (Hi-Lo Temp® Model 8700; Mallinckrodt Critical Care, Glens Falls, NY, USA).

Once snakes were within the desired temperature range, their catheter was assessed for patency by removing the catheter plug and checking for arterial blood return. If no blood flow was apparent, a saline-filled 5 ml syringe and 23 gauge needle were used to pull blood into the catheter to check for clotting. After testing for patency, catheters were flushed with 0.05 ml of saline. A 30 MBq dose of ^{99m}Tc-MAA was then injected into the catheter, followed by a 0.1 ml saline flush and 0.05 ml of heparinized saline.

Macroaggregated albumin is made up of protein particles ranging from 5 to 90 μm in size, with the majority of particles being 10–40 μm. This is the ideal size range, with the majority of particles being similar in size to the snakes' red blood cells (Lillywhite and Gallagher, 1985). When these particles reach blood vessels that have diameters too small for them to traverse they become lodged in the vessel until they are broken down into particles small enough to re-enter the circulation. Because the snakes were imaged shortly after injection, not enough time passed for the particles to degenerate.

After injection, snakes were transferred to UBC Hospital's Department of Nuclear Medicine for imaging using a General Electric Hawkeye gamma camera (https://www2.gehealthcare.com). The collimators in the camera collect gamma rays from radioisotopes and the number of gamma rays emitted along the entire length of the snakes' bodies was recorded. Snakes were imaged individually until the camera recorded approximately 250,000 gamma rays. A count of 250,000 provides sufficient resolution for statistical analysis. During imaging, a ^{99m}Tc-MAA marker was used to indicate the head, cloaca and tail tip. The technetium marker leaves visible dots at specific locations in the gamma image of the snakes' bodies. For orientation, two dots were used to indicate the head and one dot each for the cloaca and tail tip (Fig. 1).

Fig. 1. Gamma image of a garter snake taken at 6°C. Grey dots labelled A–C indicate specific anatomical features: (A) the position of the head; (B) the cloaca; and (C) the tail tip. The black arrow indicates the ^{99m}technetium-labelled macroaggregated albumin (^{99m}Tc-MAA) injection site located laterally to the snake's heart. This labelling configuration is used in all subsequent figures. The distribution of ^{99m}Tc-MAA is representative of blood flow within the body. In this case of a cold snake that has just been injected with the radioactive tracer, it can be seen that there is little blood reaching the head or tail of the snake.

Analysis

Following scanning, images of snakes were analysed using GE Xeleris computer software (https://www2.gehealthcare.com). The amount of ^{99m}Tc-MAA in the head and tail (representative of the amount of blood flow to these regions) was determined as a proportion of the total amount of ^{99m}Tc-MAA in the snake's body (Fig. 2). A Student's paired *t*-test was used to determine whether the blood flow to the snakes' heads and tails was significantly different at hot *versus* cold temperatures.

RESULTS

Snakes have significantly reduced blood flow to their tails (t=–2.71, P=0.02, d.f.=9) at low body temperatures (Fig. 3). Indeed, the ^{99m}Tc-MAA was visible at much lower counts in the snakes' tails at low temperatures (Table 1). Similarly, the snakes had significantly increased blood flow to their heads (t=2.80, P=0.02, d.f.=9) at low body temperatures; resulting in ^{99m}Tc-MAA having much higher counts in the heads at low temperatures (Table 2).

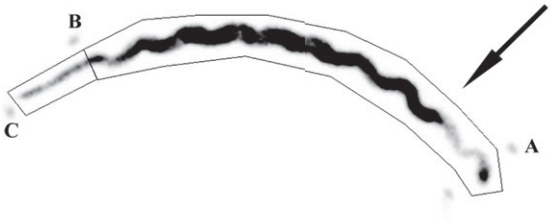

Fig. 2. Gamma image of a garter snake taken at 32°C to show how the relative distribution of blood flow to the tail was calculated. Grey dots labelled A–C indicate specific anatomical features: (A) the position of the head; (B) the cloaca; and (C) the tail tip. Polygons were used to circumscribe the area where data were collected from the body (between A and B) and the tail (between B and C) of each snake. The computer software then calculates the gamma ray counts for both polygons added together to register the gamma count for the whole snake as well as separate gamma counts from the body and the tail polygons. The relative amount of blood reaching the tail was calculated as the percentage of gamma rays from the tail. At this temperature the image indicates some blood reaching both the snake's head and tail. The same method was used to look at the proportion of gamma ray counts being emitted from the snake's head, except one polygon was drawn around the head (anterior 8 cm of the snake's body) and another polygon circumscribed the body posterior to the head.

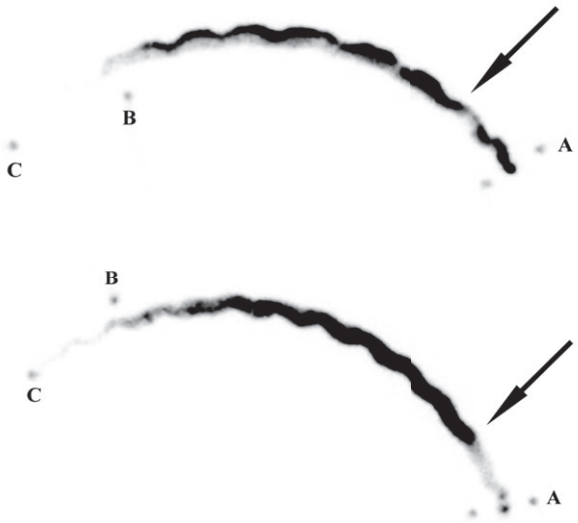

Fig. 3. Gamma images of the same snake taken at 6°C (top) and 32°C (bottom). Grey dots labelled A–C indicate specific anatomical features: (A) the position of the head; (B) the cloaca; and (C) the tail tip. At 6°C the snake has significantly less ^{99m}Tc-MAA (representative of blood flow) in its tail than at 32°C (P=0.02). Conversely, at 32°C the snake has significantly less ^{99m}Tc-MAA in its head than at 6°C (P=0.03). In comparing the two figures one sees that the snakes have a physiological mechanism to restrict blood flow to the tail at low temperatures. In contrast, at the higher temperature the tail is more extensively perfused and blood flow to the head is diminished. The actual values, as percentages of ^{99m}Tc-MAA in the tail of the snakes at high and low temperatures, are given in Table 1. These two images confirm vasomotor control of both the carotid arteries that perfuse the head and the vascular beds in the paracloacal region.

DISCUSSION

Our results confirm the assumption that snakes use regional control of blood flow to regulate body temperature. At low temperatures garter snakes have increased arterial blood flow to their heads. Conversely, at high temperatures the arterial blood flow to their heads is reduced. By regulating the flow of arterial blood to their heads, garter snakes may be able to maintain the temperature of their brains and cranial sensory organs within a narrow range for optimal functioning. At high ambient temperatures, the snakes' ability to reduce the flow of warm arterial blood to their heads may prevent their brains and sensory organs from overheating. Our results suggest that snakes can regulate their head temperatures by localized vascular control.

Additionally, garter snakes reduce arterial blood flow to their tails at low temperatures. At high temperatures the snakes increase the flow of blood to their tails. Snakes' tails have a high surface area to volume ratio and heat is expected to dissipate quickly from this region. By restricting arterial blood to their tails at low temperatures, garter snakes can mitigate total heat loss and maintain elevated body temperatures. Similarly, by allowing warm blood to enter the tail at high temperatures the snakes can shed excess heat to maintain body temperatures within their preferred range of temperatures. The paracloacal tissue appears to house an important complement of vessels with vasomotor ability that is activated, directly or indirectly, in response to temperature.

The size-dependent nature of ^{99m}Tc-MAA circulation and our decision to inject the isotope into the dorsal aorta may have had a confounding effect in our experiment. At high temperatures snakes'

Table 1. Gamma ray counts from the cloaca to the tail tip of garter snakes

	Measurements at 6–8°C			Measurements at 27–32°C		
Snake	Tail count	Total count	%*	Tail count	Total count	%*
1	5288	230614	2.24	6732	234850	2.79
2	4039	235172	1.69	10841	230513	4.49
3	3949	236598	1.64	36346	202252	15.23
4	2940	207222	1.40	23799	228537	9.43
5	554	233718	0.24	10512	231624	4.34
6	2051	236527	0.86	2558	237275	1.07
7	1791	237044	0.75	8972	232133	3.72
8	3119	241204	1.28	7285	235579	3.00
9	5760	232903	2.41	7711	230326	3.24
10	11507	222570	4.92	14615	224794	6.10

Each count represents an individual gamma ray and does not have a unit associated with the value.
*Values are percentages of the total gamma ray counts for all 10 snakes imaged.

hearts beat faster, which should increase blood pressure. However, Lillywhite and Seymour found that blood pressure in the descending aorta remains constant over a wide range of temperatures (Lillywhite and Seymour, 1978). They suggested that this stability in blood pressure is due to dilation of peripheral vascular beds at high temperatures. In terms of our experiment this suggests that at high temperatures more ^{99m}Tc-MAA is able to circulate through the body and back to the heart, eventually entering the head. Because we used a standard dose of ^{99m}Tc-MAA at both temperatures, more isotope may have been available to enter cranial circulation in the high temperature treatment. Our results, however, showed less ^{99m}Tc-MAA entering the cranial circulation at high ambient temperatures, the opposite of what is expected if more blood carrying the isotope had completed a full circulation at high temperatures. This reinforces the idea that a mechanism must exist to reduce blood flow to the head at high ambient temperatures. To remove the possible confounding effects of the location of the injection site in this experiment, a similar experiment could be done introducing ^{99m}Tc-MAA into the inferior vena cava so that the isotope would enter the cranial circulation before completing a full circuit through the body.

The androgenic hormones angiotensin (ANG) II and norepinepherine (NE) probably dictate vasomotor control of blood flow. Yung and Chiu found that exogenously administered [Val5]ANG II and NE produced a dose-dependent increase in tension in the dorsal aorta of *Naja naja* (Yung and Chiu, 1985). They also found that the dorsal aorta of *Ptyas korros* responds to NE but does not respond to ANG II, whereas the carotid artery undergoes a dose-dependent increase in tension in response to both [Val5] and [Ile5]ANG II. This suggests that different vascular beds within a snake possess different adrenergic receptors. Hence, a temperature-dependent release of ANG II could cause constriction of the carotid arteries of a snake at high temperatures, while a concurrent release of a second hormone (e.g. acetylcholine) could cause dilation of the vascular bed at the cloaca. Such a concerted dilation and contraction of vascular beds in response to different hormones would explain the preferential and dynamic regulation of blood flow that we observed.

Reducing blood flow to the tail at low ambient temperatures might be expected to reduce motor control and therefore the speed of garter snakes, making them susceptible to predation at low temperatures. However, *Thamnophis* tails are not specialized for locomotion like the tails of arboreal or aquatic genera. In fact, Jayne and Bennet have shown that garter snakes scarcely use their tails at all in locomotion (Jayne and Bennet, 1989). And, because the only organs caudal to the cloaca in garter snakes are the hemipenes in males, garter snakes do not need to constantly perfuse their tails with arterial blood. This means that garter snakes are free to use their tails for thermoregulatory control without impairing their ability to escape predators. Blood distribution at low temperatures may be different in species that have specialized tails, such as rattlesnakes that use their tails during defensive displays.

Since we only examined one species of snake living in cool climates, it is unclear whether such vasomotor adaptations are unique to this genus. Snakes that live in regions with warmer and more stable temperatures may not show the same vasomotor adaptations for thermal regulation. More generally, the phylogenetic distribution of

Table 2. Gamma ray counts from the anterior 8 cm of garter snakes, beginning at the snout

	Measurements at 6–8°C			Measurements at 27–32°C		
Snake	Head count	Body count	%*	Head count	Body count	%*
1	980	239720	0.41	4006	241449	1.66
2	32052	233988	13.70	2505	239693	1.05
3	552	240307	0.23	1175	241879	0.49
4	52279	243004	21.51	5521	239199	2.31
5	8557	240929	3.55	3108	238890	1.30
6	4136	237169	1.74	1025	242498	0.42
7	27069	240672	11.25	6434	239995	2.68
8	51342	234838	21.86	11253	242714	4.64
9	24048	245206	9.81	1654	243288	0.68
10	1035	210812	0.49	3682	252913	1.46

Each count represents an individual gamma ray and does not have a unit associated with the value.
*Values are percentages of the total gamma ray counts for all 10 snakes imaged.

vasomotor control of cranial and caudal blood flow would be worth further analysis to find out where the trait may have been lost or gained among ophidian lineages. In the cool climates occupied by the garter snakes that we studied, facultative vasoconstriction and vasodilation may complement thermoregulatory tactics, such as basking behaviour, that are common to snakes in general.

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